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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US98/26820 <b>(22) International Filing Date:</b> 17 December 1998 (17.12.98) <b>(30) Priority Data:</b> 60/068,243 19 December 1997 (19.12.97) US <b>(71) Applicant (for all designated States except US):</b> PHARMACIA & UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> GURNEY, Mark, E. [US/US]; 910 Rosewood Avenue, S.E., Grand Rapids, MI 49506 (US). LI, Jinhe [CN/US]; 8548 Old Oak Circle, Kalamazoo, MI 49009 (US). PAULEY, Adele, M. [US/US]; 213 E. Brighton, Plainwell, MI 49080 (US). <b>(74) Agent:</b> KERBER, Lori, L.; Pharmacia & Upjohn Company, Intellectual Property Legal Services, 301 Henrietta Street, Kalamazoo, MI 49001 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> HUMAN SEL-10 POLYPEPTIDES AND POLYNUCLEOTIDES THAT ENCODE THEM <b>(57) Abstract</b> <p>The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding either of two alternative splice variants of human sel-10, one of which is expressed in hippocampal cells, and one of which is expressed in mammary cells. The invention also provides isolated sel-10 polypeptides and cell lines which express them in which A<math>\beta</math> processing is altered.</p>		

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## Human Sel-10 Polypeptides and Polynucleotides that Encode Them

### FIELD OF THE INVENTION

5           The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding either of two alternative splice variants of human sel-10, one of which is expressed in hippocampal cells, and one of which is expressed in mammary cells. The invention also provides isolated sel-10 polypeptides.

### 10    BACKGROUND OF THE INVENTION

          Alzheimer's disease (AD) is a degenerative disorder of the central nervous system which causes progressive memory and cognitive decline during mid to late adult life. The disease is accompanied by a wide range of neuropathologic features including extracellular  
15    amyloid plaques and intra-neuronal neurofibrillary tangles. (Sherrington, R., *et al.*; *Nature* 375: 754-60 (1995)). Although the pathogenic pathway leading to AD is not well understood, several genetic loci are known to be involved in the development of the disease.

          Genes associated with early onset Alzheimer's disease (AD) have been identified by the use of mapping studies in families with early-onset AD. These studies have shown that  
20    genetic loci on chromosomes 1 and 14 were likely to be involved in AD. Positional cloning of the chromosome 14 locus identified a novel mutant gene encoding an eight-transmembrane domain protein which subsequently was named presenilin-1 (PS-1). (Sherrington, R., *et al.*; *Nature* 375: 754-60 (1995)). Blast search of the human EST database revealed a single EST exhibiting homology to PS-1, designated presenilin-2 (PS-2) which was shown to be the gene  
25    associated with AD on chromosome 1. (Levy-Lahad, E. *et al.*, *Science* 269:973-977 (1995); Rogaev, E. I., *et al.*, *Nature* 376: 775-8 (1995); Li, J. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 92: 12180-12184 (1995)).

          Mutations in PS-1 and PS-2 that are associated with Alzheimer's disease are primarily missense mutations. Both PS-1 and PS-2 undergo proteolytic processing, which can be  
30    altered by the point mutations found in familial Alzheimer's disease [Perez-Tur, J. *et al.*, *Neuroreport* 7: 297-301 (1995); Mercken, M. *et al.*, *FEBS Lett.* 389: 297-303 (1996)]. PS-1 gene expression is widely distributed across tissues, while the highest levels of PS-2 mRNA are found in pancreas and skeletal muscle. (Li, J. *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 92:

12180-12184 (1995); Jinhe Li, personal communication). The highest levels of PS-2 protein, however, are found in brain (Jinhe Li, personal communication). Both PS-1 and PS-2 proteins have been localized to the endoplasmic reticulum, the Golgi apparatus, and the nuclear envelope. (Jinhe Li, personal communication; Kovacs, D.M. *et al.*, *Nat. Med.* 2:224-229 (1996); Doan, A. *et al.*, *Neuron* 17: 1023-1030 (1996)). Mutations in either the PS-1 gene or the PS-2 gene alter the processing of the amyloid protein precursor (APP) such that the ratio of A-beta<sub>1-42</sub> is increased relative to A-beta<sub>1-40</sub> (Scheuner, D. *et al.*, *Nat. Med.* 2: 864-870 (1996)). When coexpressed in transgenic mice with human APP, a similar increase in the ratio of A-beta<sub>1-42</sub> as compared to A-beta<sub>1-40</sub> is observed (Borchelt, D. R. *et al.*, *Neuron* 17: 1005-1013 (1996); Citron, M. *et al.*, *Nat. Med.* 3: 67-72 (1997); Duff, K. *et al.*, *Nature* 383: 710-713 (1996)), together with an acceleration of the deposition of A-beta in amyloid plaques (Borchelt *et al.*, *Neuron* 19: 939 (1997)).

Despite the above-described observations made with respect to the role of PS-1 and PS-2 in AD, their biological function remains unknown, placing them alongside a large number of human disease genes having an unknown biological function. Where the function of a gene or its product is unknown, genetic analysis in model organisms can be useful in placing such genes in known biochemical or genetic pathways. This is done by screening for extragenic mutations that either suppress or enhance the effect of mutations in the gene under analysis. For example, extragenic suppressors of loss-of-function mutations in a disease gene may turn on the affected genetic or biochemical pathway downstream of the mutant gene, while suppressors of gain-of-function mutations will probably turn the pathway off.

One model organism that can be used in the elucidation of the function of the presenilin genes is *C. elegans*, which contains three genes having homology to PS-1 and PS-2, with *sel-12* having the highest degree of homology to the genes encoding the human presenilins. *Sel-12* was discovered in a screen for genetic suppressors of an activated notch receptor, *lin-12(d)* (Levitan, D. *et al.*, *Nature* 377: 351-354 (1995)). *Lin-12* functions in development to pattern cell lineages. Hypermorphic mutations such as *lin-12(d)*, which increase *lin-12* activity, cause a "multi-vulval" phenotype, while hypomorphic mutations which decrease activity cause eversion of the vulva, as well as homeotic changes in several other cell lineages (Greenwald, I., *et al.*, *Nature* 346: 197-199 (1990); Sundaram, M. *et al.*, *Genetics* 135: 755-763 (1993)). *Sel-12* mutations suppress hypermorphic *lin-12(d)* mutations, but only if the *lin-12(d)* mutations activate signaling by the intact *lin-12(d)* receptor (Levitan, D. *et al.*, *Nature* 377: 351-354 (1995)). *Lin-12* mutations that truncate the cytoplasmic

domain of the receptor also activate signaling (Greenwald, I., *et al.*, *Nature* 346: 197-199 (1990)), but are not suppressed by mutations of *sel-12* (Levitan, D. *et al.*, *Nature* 377: 351-354 (1995)). This implies that *sel-12* mutations act upstream of the *lin-12* signaling pathway, perhaps by decreasing the amount of functional *lin-12* receptor present in the plasma  
5 membrane. In addition to suppressing certain *lin-12* hypermorphic mutations, mutations to *sel-12* cause a loss-of-function for egg laying, and thus internal accumulation of eggs, although the mutants otherwise appear anatomically normal (Levitan, D. *et al.*, *Nature* 377: 351-354 (1995)). *Sel-12* mutants can be rescued by either human PS-1 or PS-2, indicating that *sel-12*, PS-1 and PS-2 are functional homologues (Levitan, D., *et al.*, *Proc. Natl. Acad.*  
10 *Sci. U.S.A* 93: 14940-14944 (1996)).

A second gene, *sel-10*, has been identified in a separate genetic screen for suppressors of *lin-12* hypomorphic mutations. Loss-of-function mutations in *sel-10* restore signaling by *lin-12* hypomorphic mutants. As the lowering of *sel-10* activity elevates *lin-12* activity, it can be concluded that *sel-10* acts as a negative regulator of *lin-12* signaling. *Sel-10* also acts as a  
15 negative regulator of *sel-12*, the *C. elegans* presenilin homologue (Levy-Lahad, E. *et al.*, *Science* 269:973-977 (1995)). Loss of *sel-10* activity suppresses the egg laying defect associated with hypomorphic mutations in *sel-12* (Iva Greenwald, personal communication). The effect of loss-of-function mutations to *sel-10* on *lin-12* and *sel-12* mutations indicates that *sel-10* acts as a negative regulator of both *lin-12/notch* and presenilin activity. Thus, a human  
20 homologue of *C. elegans sel-10* would be expected to interact genetically and/or physiologically with human presenilin genes in ways relevant to the pathogenesis of Alzheimer's Disease.

In view of the foregoing, it will be clear that there is a continuing need for the identification of genes related to AD, and for the development of assays for the identification  
25 of agents capable of interfering with the biological pathways that lead to AD.

### INFORMATION DISCLOSURE

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10 Greenwald-I (1996) Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U.S.A.* 93: 14940-4.

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Sundaram-M; Greenwald-I (1993) Genetic and phenotypic studies of hypomorphic *lin-12* mutants in *Caenorhabditis elegans*. *Genetics*. 135: 755-63.

F55B12.3 GenPep Report (WMBL locus CEF55B12, accession z79757).

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WO 97/11956

## SUMMARY OF THE INVENTION

The present invention provides isolated nucleic acid molecules comprising a  
25 polynucleotide encoding human sel-10, which is expressed in hippocampal cells and in mammary cells. Unless otherwise noted, any reference herein to sel-10 will be understood to refer to human sel-10, and to encompass both hippocampal and mammary sel-10. Fragments of hippocampal sel-10 and mammary sel-10 are also provided.

In a preferred embodiment, the invention provides an isolated nucleic acid molecule  
30 comprising a polynucleotide having a sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a human sel-10 polypeptide having the complete amino acid sequence selected from the group consisting of SEQ ID NO:3,



SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, or as encoded by the cDNA clone contained in ATCC Deposit No.98978;

- (b) a nucleotide sequence encoding a human sel-10 polypeptide having the complete amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10, or as encoded by the cDNA clone contained in ATCC Deposit No. 98979; and

(c) a nucleotide sequence complementary to the nucleotide sequence of (a) or (b).

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent conditions to a polynucleotide encoding sel-10, or fragments thereof.

The present invention also provides vectors comprising the isolated nucleic acid molecules of the invention, host cells into which such vectors have been introduced, and recombinant methods of obtaining a sel-10 polypeptide comprising culturing the above-described host cell and isolating the sel-10 polypeptide.

In another aspect, the invention provides isolated sel-10 polypeptides, as well as fragments thereof. In a preferred embodiment, the sel-10 polypeptides have an amino acid sequence selected from the group consisting of SEQ ID NO:3, 4, 5, 6, 7, 8, 9, and 10. Isolated antibodies, both polyclonal and monoclonal, that bind specifically to sel-10 polypeptides are also provided.

## BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B: Figures 1A and 1B are western blots showing protein expression in HEK293 cells transfected with PS1-C-FLAG, 6-myc-N-sel-10, and APP695NL-KK cDNAs.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding human sel-10. The nucleotide sequence of human hippocampal sel-10 (hhsel-10), which sequence is given in SEQ ID NO:1, encodes five hhsel-10 polypeptides (hhsel-10-(1), hhsel-10-(2), hhsel-10-(3), hhsel-10-(4), and hhsel-10-(5), referred to collectively herein as hhsel-10). The nucleotide sequence of human mammary sel-10

(hmsel-10), which sequence is given in SEQ ID NO:2, encodes three hmsel-10 polypeptides (hmSel-10-(1), hmSel-10-(2), and hmsel-10-(3), referred to collectively herein as hmsel-10). The nucleotide sequences of the hhsel-10 polynucleotides are given in SEQ ID NO. 1, where nucleotide residues 45-1928 of SEQ ID NO. 1 correspond to hhsel-10-(1), nucleotide residues  
5 150-1928 of SEQ ID NO. 1 correspond to hhsel-10-(2), nucleotide residues 267-1928 of SEQ ID NO. 1 correspond to hhsel-10-(3), nucleotide residues 291-1928 of SEQ ID NO. 1 correspond to hhsel-10-(4), and nucleotide residues 306-1928 of SEQ ID NO. 1 correspond to hhsel-10-(5). The nucleotide sequences of the hmSel-10 polynucleotides are given in SEQ ID NO. 2, where nucleotide residues 180-1949 of SEQ ID NO. 2 correspond to hmSel-10-(1),  
10 nucleotide residues 270-1949 of SEQ ID NO. 2 correspond to hmSel-10-(2), and nucleotide residues 327-1949 of SEQ ID NO. 2 correspond to hmSel-10-(3). The amino acid sequences of the polypeptides encoded by the hhsel-10 and hmSel-10 nucleic acid molecules are given as follows: SEQ ID NOS: 3, 4, 5, 6, and 7 correspond to the hhsel-10-(1), hhsel-10-(2), hhsel-10-(3), hhsel-10-(4), and hhsel-10-(5) polypeptides, respectively, and SEQ ID NOS: 8,  
15 9, and 10 correspond to the hmSel-10-(1), hmSel-10-(2), and hmSel-10-(3) polypeptides, respectively. Unless otherwise noted, any reference herein to sel-10 will be understood to refer to human sel-10, and to encompass all of the hippocampal and mammary sel-10 nucleic acid molecules (in the case of reference to sel-10 nucleic acid, polynucleotide, DNA, RNA, or gene) or polypeptides (in the case of reference to sel-10 protein, polypeptide, amino acid  
20 sequence). Fragments of hippocampal sel-10 and mammary sel-10 nucleic acid molecules and polypeptides are also provided.

The nucleotide sequence of SEQ ID NO:1 was obtained as described in Example 1, and is contained in cDNA clone PNV 102-1, which was deposited on November 9, 1998, at the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110, and  
25 given accession number 98978. The nucleotide sequence of SEQ ID NO:2 was obtained as described in Example 1, and is contained in cDNA clone PNV 108-2, which was deposited on November 9, 1998, at the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110, and given accession number 98979.

The human sel-10 polypeptides of the invention share homology with *C. elegans* sel-  
30 10, as well as with members of the  $\beta$ -transducin protein family, including yeast CDC4, and human LIS-1. This family is characterized by the presence of an F-box and multiple WD-40 repeats (Li, J., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 92:12180-12184 (1995)). The repeats are 20-40 amino acids long and are bounded by gly-his (GH) and trp-asp (WD) residues. The

three dimensional structure of  $\beta$ -transducin indicates that the WD40 repeats form the arms of a seven-bladed propeller like structure (Sondek, J., *et al.*, *Nature* 379:369-374 (1996)). Each blade is formed by four alternating pleats of beta-sheet with a pair of the conserved aspartic acid residues in the protein motif forming the limits of one internal beta strand. WD40 repeats are found in over 27 different proteins which represent diverse functional classes (Neer, E.J., *et al.*, *Nature* 371:297-300 (1994)). These regulate cellular functions including cell division, cell fate determination, gene transcription, signal transduction, protein degradation, mRNA modification and vesicle fusion. This diversity in function has led to the hypothesis that  $\beta$ -transducin family members provide a common scaffolding upon which multiprotein complexes can be assembled.

The nucleotide sequence given in SEQ ID NO:1 corresponds to the nucleotide sequence encoding hhsel-10, while the nucleotide sequence given in SEQ ID NO:2 corresponds to the nucleotide sequence encoding hmsel-10. The isolation and sequencing of DNA encoding sel-10 is described below in Examples 1 and 2.

As is described in Examples 1 and 2, automated sequencing methods were used to obtain the nucleotide sequence of sel-10. The sel-10 nucleotide sequences of the present invention were obtained for both DNA strands, and are believed to be 100% accurate. However, as is known in the art, nucleotide sequence obtained by such automated methods may contain some errors. Nucleotide sequences determined by automation are typically at least about 90%, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of a given nucleic acid molecule. The actual sequence may be more precisely determined using manual sequencing methods, which are well known in the art. An error in sequence which results in an insertion or deletion of one or more nucleotides may result in a frame shift in translation such that the predicted amino acid sequence will differ from that which would be predicted from the actual nucleotide sequence of the nucleic acid molecule, starting at the point of the mutation. The sel-10 DNA of the present invention includes cDNA, chemically synthesized DNA, DNA isolated by PCR, genomic DNA, and combinations thereof. Genomic sel-10 DNA may be obtained by screening a genomic library with the sel-10 cDNA described herein, using methods that are well known in the art. RNA transcribed from sel-10 DNA is also encompassed by the present invention.

Due to the degeneracy of the genetic code, two DNA sequences may differ and yet encode identical amino acid sequences. The present invention thus provides isolated nucleic acid molecules having a polynucleotide sequence encoding any of the sel-10 polypeptides of

the invention, wherein said polynucleotide sequence encodes a sel-10 polypeptide having the complete amino acid sequence of SEQ ID NOs:3-10, or fragments thereof.

Also provided herein are purified sel-10 polypeptides, both recombinant and non-recombinant. Variants and derivatives of native sel-10 proteins that retain any of the biological activities of sel-10 are also within the scope of the present invention. As is described above, the sel-10 polypeptides of the present invention share homology with yeast CDC4. As CDC4 is known to catalyze ubiquitination of specific cellular proteins (Feldman *et al.*, *Cell* 91:221 (1997)), it may be inferred that sel-10 will also have this activity. Assay procedures for demonstrating such activity are well known, and involve reconstitution of the ubiquitinating system using purified human sel-10 protein together with the yeast proteins Cdc4p, Cdc53p and Skp1p, or their human orthologs, and an E1 enzyme, the E2 enzyme Cdc34p or its human ortholog, ubiquitin, a target protein and an ATP regenerating system (Feldman *et al.*, 1997). Skp1p associates with Cdc4p through a protein domain called an F-box (Bai *et al.*, *Cell* 86:263 (1996)). The F-box protein motif is found in yeast CDC4, *C. elegans* sel-10, mouse sel-10 and human sel-10. The sel-10 ubiquitination system may be reconstituted with the *C. elegans* counterparts of the yeast components, *e.g.*, cul-1 (also known as lin-19) protein substituting for Cdc53p (Kipreos *et al.*, *Cell* 85:829 (1996)) and the protein F46A9 substituting for Skp1p, or with their mammalian counterparts, *e.g.*, Cul-2 protein substituting for Cdc53p (Kipreos *et al.*, 1996) and mammalian Skp1p substituting for yeast Skp1p. A phosphorylation system provided by a protein kinase is also included in the assay system as per Feldman *et al.*, 1997.

Sel-10 variants may be obtained by mutation of native sel-10-encoding nucleotide sequences, for example. A sel-10 variant, as referred to herein, is a polypeptide substantially homologous to a native sel-10 but which has an amino acid sequence different from that of native sel-10 because of one or more deletions, insertions, or substitutions in the amino acid sequence. The variant amino acid or nucleotide sequence is preferably at least about 80% identical, more preferably at least about 90% identical, and most preferably at least about 95% identical, to a native sel-10 sequence. Thus, a variant nucleotide sequence which contains, for example, 5 point mutations for every one hundred nucleotides, as compared to a native sel-10 gene, will be 95% identical to the native protein. The percentage of sequence identity, also termed homology, between a native and a variant sel-10 sequence may also be determined, for example, by comparing the two sequences using any of the computer programs commonly employed for this purpose, such as the Gap program (Wisconsin Sequence Analysis Package,

Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wisconsin), which uses the algorithm of Smith and Waterman (*Adv. Appl. Math.* 2: 482-489 (1981)).

Alterations of the native amino acid sequence may be accomplished by any of a number of known techniques. For example, mutations may be introduced at particular locations by procedures well known to the skilled artisan, such as oligonucleotide-directed mutagenesis, which is described by Walder *et al.* (*Gene* 42:133 (1986)); Bauer *et al.* (*Gene* 37:73 (1985)); Craik (*BioTechniques*, January 1985, pp. 12-19); Smith *et al.* (*Genetic Engineering: Principles and Methods*, Plenum Press (1981)); and U.S. Patent Nos. 4,518,584 and 4,737,462.

Sel-10 variants within the scope of the invention may comprise conservatively substituted sequences, meaning that one or more amino acid residues of a sel-10 polypeptide are replaced by different residues that do not alter the secondary and/or tertiary structure of the sel-10 polypeptide. Such substitutions may include the replacement of an amino acid by a residue having similar physicochemical properties, such as substituting one aliphatic residue (Ile, Val, Leu or Ala) for another, or substitution between basic residues Lys and Arg, acidic residues Glu and Asp, amide residues Gln and Asn, hydroxyl residues Ser and Tyr, or aromatic residues Phe and Tyr. Further information regarding making phenotypically silent amino acid exchanges may be found in Bowie *et al.*, *Science* 247:1306-1310 (1990). Other sel-10 variants which might retain substantially the biological activities of sel-10 are those where amino acid substitutions have been made in areas outside functional regions of the protein.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent conditions to a portion of the nucleic acid molecules described above, *e.g.*, to at least about 15 nucleotides, preferably to at least about 20 nucleotides, more preferably to at least about 30 nucleotides, and still more preferably to at least about from 30 to at least about 100 nucleotides, of one of the previously described nucleic acid molecules. Such portions of nucleic acid molecules having the described lengths refer to, *e.g.*, at least about 15 contiguous nucleotides of the reference nucleic acid molecule. By stringent hybridization conditions is intended overnight incubation at about 42°C for about 2.5 hours in 6 X SSC/0.1% SDS, followed by washing of the filters in 1.0 X SSC at 65°C, 0.1% SDS.

Fragments of the sel-10-encoding nucleic acid molecules described herein, as well as polynucleotides capable of hybridizing to such nucleic acid molecules may be used as a probe or as primers in a polymerase chain reaction (PCR). Such probes may be used, *e.g.*, to detect the presence of *sel-10* nucleic acids in *in vitro* assays, as well as in Southern and northern blots. Cell types expressing sel-10 may also be identified by the use of such probes. Such procedures are well known, and the skilled artisan will be able to choose a probe of a length suitable to the particular application. For PCR, 5' and 3' primers corresponding to the termini of a desired sel-10 nucleic acid molecule are employed to isolate and amplify that sequence using conventional techniques.

Other useful fragments of the sel-10 nucleic acid molecules are antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence capable of binding to a target sel-10 mRNA (using a sense strand), or sel-10 DNA (using an antisense strand) sequence.

In another aspect, the invention includes sel-10 polypeptides with or without associated native pattern glycosylation. Sel-10 expressed in yeast or mammalian expression systems (discussed below) may be similar to or significantly different from a native sel-10 polypeptide in molecular weight and glycosylation pattern. Expression of sel-10 in bacterial expression systems will provide non-glycosylated sel-10.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. Sel-10 polypeptides may be recovered and purified from recombinant cell cultures by well-known methods, including ammonium sulfate or ethanol precipitation, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. In a preferred embodiment, high performance liquid chromatography (HPLC) is employed for purification.

The present invention also relates to vectors comprising the polynucleotide molecules of the invention, as well as host cell transformed with such vectors. Any of the polynucleotide molecules of the invention may be joined to a vector, which generally includes a selectable marker and an origin of replication, for propagation in a host. Because the invention also provides sel-10 polypeptides expressed from the polynucleotide molecules described above, vectors for the expression of sel-10 are preferred. The vectors include DNA encoding any of the sel-10 polypeptides described above or below, operably linked to suitable transcriptional or translational regulatory sequences, such as those derived from a mammalian, microbial,

viral, or insect gene. Examples of regulatory sequences include transcriptional promoters, operators, or enhancers, mRNA ribosomal binding sites, and appropriate sequences which control transcription and translation. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the DNA encoding sel-10. Thus, a promoter  
5 nucleotide sequence is operably linked to a sel-10 DNA sequence if the promoter nucleotide sequence directs the transcription of the sel-10 sequence.

Selection of suitable vectors to be used for the cloning of polynucleotide molecules encoding sel-10, or for the expression of sel-10 polypeptides, will of course depend upon the host cell in which the vector will be transformed, and, where applicable, the host cell from  
10 which the sel-10 polypeptide is to be expressed. Suitable host cells for expression of sel-10 polypeptides include prokaryotes, yeast, and higher eukaryotic cells, each of which is discussed below.

The sel-10 polypeptides to be expressed in such host cells may also be fusion proteins which include regions from heterologous proteins. Such regions may be included to allow,  
15 *e.g.*, secretion, improved stability, or facilitated purification of the polypeptide. For example, a sequence encoding an appropriate signal peptide can be incorporated into expression vectors. A DNA sequence for a signal peptide (secretory leader) may be fused in-frame to the sel-10 sequence so that sel-10 is translated as a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cell promotes extracellular secretion of  
20 the sel-10 polypeptide. Preferably, the signal sequence will be cleaved from the sel-10 polypeptide upon secretion of sel-10 from the cell. Non-limiting examples of signal sequences that can be used in practicing the invention include the yeast I-factor and the honeybee melatin leader in sf9 insect cells.

In a preferred embodiment, the sel-10 polypeptide will be a fusion protein which  
25 includes a heterologous region used to facilitate purification of the polypeptide. Many of the available peptides used for such a function allow selective binding of the fusion protein to a binding partner. For example, the sel-10 polypeptide may be modified to comprise a peptide to form a fusion protein which specifically binds to a binding partner, or peptide tag. Non-limiting examples of such peptide tags include the 6-His tag, thioredoxin tag, FLAG tag,  
30 hemagglutinin tag, GST tag, and OmpA signal sequence tag. As will be understood by one of skill in the art, the binding partner which recognizes and binds to the peptide may be any molecule or compound including metal ions (*e.g.*, metal affinity columns), antibodies, or fragments thereof, and any protein or peptide which binds the peptide. These tags may be

recognized by fluorescein or rhodamine labeled antibodies that react specifically with each type of tag

Suitable host cells for expression of sel-10 polypeptides include prokaryotes, yeast, and higher eukaryotic cells. Suitable prokaryotic hosts to be used for the expression of sel-10 include bacteria of the genera *Escherichia*, *Bacillus*, and *Salmonella*, as well as members of the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. For expression in, e.g., *E. coli*, a sel-10 polypeptide may include an N-terminal methionine residue to facilitate expression of the recombinant polypeptide in a prokaryotic host. The N-terminal Met may optionally then be cleaved from the expressed sel-10 polypeptide.

Expression vectors for use in prokaryotic hosts generally comprise one or more phenotypic selectable marker genes. Such genes generally encode, e.g., a protein that confers antibiotic resistance or that supplies an auxotrophic requirement. A wide variety of such vectors are readily available from commercial sources. Examples include pSPORT vectors, pGEM vectors (Promega), pPROEX vectors (LTI, Bethesda, MD), Bluescript vectors (Stratagene), and pQE vectors (Qiagen).

Sel-10 may also be expressed in yeast host cells from genera including *Saccharomyces*, *Pichia*, and *Kluveromyces*. Preferred yeast hosts are *S. cerevisiae* and *P. pastoris*. Yeast vectors will often contain an origin of replication sequence from a 2T yeast plasmid, an autonomously replicating sequence (ARS), a promoter region, sequences for polyadenylation, sequences for transcription termination, and a selectable marker gene.

Vectors replicable in both yeast and *E. coli* (termed shuttle vectors) may also be used. In addition to the above-mentioned features of yeast vectors, a shuttle vector will also include sequences for replication and selection in *E. coli*. Direct secretion of sel-10 polypeptides expressed in yeast hosts may be accomplished by the inclusion of nucleotide sequence encoding the yeast I-factor leader sequence at the 5' end of the sel-10-encoding nucleotide sequence.

Insect host cell culture systems may also be used for the expression of Sel-10 polypeptides. In a preferred embodiment, the sel-10 polypeptides of the invention are expressed using a baculovirus expression system. Further information regarding the use of baculovirus systems for the expression of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988).

In another preferred embodiment, the sel-10 polypeptide is expressed in mammalian host cells. Non-limiting examples of suitable mammalian cell lines include the COS-7 line of



monkey kidney cells (Gluzman *et al.*, *Cell* 23:175 (1981)) and Chinese hamster ovary (CHO) cells.

The choice of a suitable expression vector for expression of the sel-10 polypeptides of the invention will of course depend upon the specific mammalian host cell to be used, and is within the skill of the ordinary artisan. Examples of suitable expression vectors include pcDNA3 (Invitrogen) and pSVL (Pharmacia Biotech). Expression vectors for use in mammalian host cells may include transcriptional and translational control sequences derived from viral genomes. Commonly used promoter sequences and enhancer sequences which may be used in the present invention include, but are not limited to, those derived from human cytomegalovirus (CMV), Adenovirus 2, Polyoma virus, and Simian virus 40 (SV40). Methods for the construction of mammalian expression vectors are disclosed, for example, in Okayama and Berg (*Mol. Cell. Biol.* 3:280 (1983)); Cosman *et al.* (*Mol. Immunol.* 23:935 (1986)); Cosman *et al.* (*Nature* 312:768 (1984)); EP-A-0367566; and WO 91/18982.

The polypeptides of the present invention may also be used to raise polyclonal and monoclonal antibodies, which are useful in diagnostic assays for detecting sel-10 polypeptide expression. Such antibodies may be prepared by conventional techniques. See, for example, *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988); *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet *et al.* (eds.), Plenum Press, New York (1980).

The sel-10 nucleic acid molecules of the present invention are also valuable for chromosome identification, as they can hybridize with a specific location on a human chromosome. There is a current need for identifying particular sites on the chromosome, as few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. The relationship between genes and diseases that have been mapped to the same chromosomal region can then be identified through linkage analysis, wherein the coinheritance of physically adjacent genes is determined. Whether a gene appearing to be related to a particular disease is in fact the cause of the disease can then be determined by comparing the nucleic acid sequence between affected and unaffected individuals.

The sel-10 polypeptides of the invention, and the DNA encoding them, may also be used to further elucidate the biological mechanism of AD, and may ultimately lead to the

identification of compounds that can be used to alter such mechanisms. The sel-10 polypeptides of the invention are 47.6% identical and 56.7% similar to *C. elegans* sel-10. As is described above, mutations to *C. elegans* sel-10 are known to suppress mutations to sel-12 that result in a loss-of-function for egg laying, and also to suppress certain hypomorphic mutations to *lin-12*. Mutations to *C. elegans* sel-12 can also be rescued by either of the human AD-linked genes PS-1 (42.7% identical to sel-12) or PS-2 (43.4% identical to sel-12). However, human PS-1 with a familial AD-linked mutant has a reduced ability to rescue sel-12 mutants (Levitan, D. *et al.*, *Proc. Natl. Acad. Sci. USA* 93: 14940-14944 (1996)).

This demonstrated interchangeability of human and *C. elegans* genes in the notch signaling pathway makes it reasonable to predict that mutations of human sel-10 will suppress mutations to PS-1 or PS-2 that lead to AD, especially in light of the predicted structure of sel-10. As described above, PS-1 and PS-2 mutations that lead to AD are those which interfere with the proteolytic processing of PS-1 or PS-2. The sel-10 polypeptides of the invention are members of the  $\beta$ -transducin protein family, which includes yeast CDC4, a component of an enzyme which functions in the ubiquitin-dependent protein degradation pathway. Thus, human sel-10 may regulate presenilin degradation via the ubiquitin-proteasome pathway. Alternatively, or in addition, human sel-10 may alter presenilin function by targeting for degradation through ubiquitination a modulator of presenilin activity, e.g., a negative regulator. Therefore, mutations to sel-10 may reverse the faulty proteolytic processing of PS-1 or PS-2 which occurs as a result of mutation to PS-1 or PS-2 or otherwise increase presenilin function. For the same reason, inhibition of sel-10 activity may also act to reverse PS-1 or PS-2 mutations. Thus, it may be hypothesized that compounds which inhibit either the expression or the activity of the human sel-10 polypeptides of the invention may reverse the effects of mutations to PS-1 or PS-2, and thus be useful for the prevention or treatment of AD.

Thus, *C. elegans* may be used as a genetic system for the identification of agents capable of inhibiting the activity or expression of the human sel-10 polypeptides of the invention. A suitable *C. elegans* strain for use in such assays lacks a gene encoding active *C. elegans* sel-10, and exhibits a loss-of-function for egg-laying resulting from an inactivated sel-12 gene. Construction of *C. elegans* strains having a loss-of-function for egg-laying due to mutation of sel-12 may be accomplished using routine methods, as both the sequence of sel-12 (Genebank accession number U35660) and mutations to sel-12 resulting in a loss-of-function for egg laying are known (*see* Levitan *et al.*, *Nature* 377: 351-354 (1995), which

describes construction of *C. elegans sel-12(ar171)*). An example of how to make such a strain is also given in Levitan *et al.* (*Nature* 377: 351-354 (1995)). Wild-type *C. elegans sel-10* in the *C. elegans sel-12(ar171)* , is also mutagenized using routine methods, such as the technique used for *sel-12* mutagenesis in Levitan *et al.*, *supra*.

5 In order to identify compounds inhibiting human *sel-10* activity, a DNA vector containing a human *sel-10* gene encoding any of the wild-type human *sel-10* proteins of the invention is introduced into the above-described *C. elegans* strain. In a preferred embodiment, the heterologous human *sel-10* gene is integrated into the *C. elegans* genome. The gene is then expressed, using techniques described in Levitan *et al.* (*Proc. Natl. Acad. Sci. USA* 93:  
10 14940-14944 (1996)). Test compounds are then administered to this strain in order to determine whether a given agent is capable of inhibiting *sel-10* activity so as to suppress mutations to *sel-12* or *lin-12* that result in egg-laying defects. Egg-laying in this strain is then determined, e.g. by the assay described in Levitan *et al.* (*Proc. Natl. Acad. Sci. USA* 93:  
14940-14944 (1996)). To confirm that the compound's effect on egg-laying is due to  
15 inhibition of *sel-10* activity, the action of the compound can be tested in a second biochemical or genetic pathway that is known to be affected by loss-of-function mutations in *sel-10* (e.g., further elevation of *lin-12* activity in *lin-12(d)* hypomorphic strains). Such assays may be performed as described in Sundarem and Greenwald (*Genetics* 135: 765-783 (1993)).

Alternatively, compounds are tested for their ability to inhibit the E3 Ubiquitin  
20 Ligating Enzyme. Assays procedures for demonstrating such activity are well known, and involve reconstitution of the ubiquitinating system using purified human *sel-10* protein together with the yeast proteins Cdc4p, Cdc53p and Skp1p and an E1 enzyme, the E2 enzyme Cdc34p, ubiquitin, a target protein and an ATP regenerating system (Feldman *et al.*, 1997). The *sel-10* ubiquitination system may also be reconstituted with the *C. elegans* counterparts of  
25 the yeast components, e.g., *cul-1* (also known as *lin-19*) protein substituting for Cdc53p (Kipreos *et al.*, *Cell* 85:829 (1996)) and the protein F46A9 substituting for Skp1p, or with their mammalian counterparts, e.g., Cul-2 protein substituting for Cdc53p (Kipreos *et al.*, *ibid.*) and mammalian Skp1p substituting for yeast Skp1p. A phosphorylation system provided by a protein kinase is also to be included in the assay system as per Feldman *et al.*,  
30 1997.

Alternatively, cell lines which express human *sel-10* due to transformation with a human *sel-10* cDNA and which as a consequence have elevated APP processing and formation of A $\beta$ <sub>1-40</sub> or A $\beta$ <sub>1-42</sub> may also be used for such assays as in Example 3. Compounds

may be tested for their ability to reduce the elevated A $\beta$  processing seen in the sel-10 transformed cell line.

Compounds that rescue the egg-laying defect or that inhibit E3 Ubiquitin Ligating Enzyme are then screened for their ability to cause a reduction in the production of A-beta<sub>1-40</sub> or A-beta<sub>1-42</sub> in a human cell line. Test compounds are used to expose IMR-32 or other human cell lines known to produce A-beta<sub>1-40</sub> or A-beta<sub>1-42</sub> (Asami-Okada *et al.*, *Biochemistry* 34: 10272-10278 (1995)), or in human cell lines engineered to express human APP at high levels. In these assays, A-beta<sub>1-40</sub> or A-beta<sub>1-42</sub> is measured in cell extracts or after release into the medium by ELISA or other assays which are known in the art (Borchelt *et al.*, *Neuron* 17: 1005-1013 (1996); Citron *et al.*, *Nat. Med.* 3: 67-72 (1997)).

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

15

## EXAMPLES

### *Example 1: Identification of a human homologue to C. elegans sel-10*

#### *Results*

20 *Identification of sel-10 in ACEDB:* Sel-10 maps between the cloned polymorphisms arP3 and TCPARI just to the left of him-5 [ACEDB entry wm95p536]. Three phage lambda clones have been sequenced across the interval, F53C11, F09F3, and F55B12. *Sel-10* is reported to have homology to yeast *cdc4* [ACEDB entry wm97ab259]. Blast search revealed a single ORF with homology to yeast *cdc4* (CC4\_YST) within the interval defined by arP3 and TCPARI corresponding to the GenPep entry F55B12.3. F55B12.3, like yeast *cdc4*, is a member of the  $\beta$ -transducin protein family. This family is characterized by the presence of multiple WD40 repeats [Neer, E.J. *et al.*, *Nature* 371: 297-300 (1994)].

*Identification of a human sel-10 homologue, Incyte 028971:* The GenPep entry F55B12.3 was used to search the LifeSeq, LifeSeq FL and EMBL data bases using tblastn. The search revealed multiple homologies to  $\beta$ -transducin family members including LIS-1 (S36113 and P43035), a gene implicated in Miller-Dieker lissencephaly, a *Xenopus laevis* gene, TRCPXEN (U63921), and a human contig in LifeSeq FL, 028971. Since there also are

multiple  $\beta$ -transducin family members within the *C. elegans* genome, these were collected using multiple blast searches and then clustered with the *sel-10* candidate genes. Multiple alignments were performed with the DNASTar program Megalign using the Clustal method. This revealed that LIS-1 clustered with T03F6.F, a different  $\beta$ -transducin family member and  
5 thus excluded it as a candidate *sel-10* homologue. TRCPXEN clustered with K10B2.1, a gene which also clusters with F55B12.3 and CC4YST, while Incyte 028971 clustered with *sel-10*. Thus, Incyte 028971 appears to encode the human homologue of *C. elegans sel-10*. Sequence homology between *sel-10* and 028971 is strongest in the region of the protein containing 7 repeats of the WD40 motif. The Incyte 028971 contig contains 44 ESTs from  
10 multiple libraries including pancreas, lung, T-lymphocytes, fibroblasts, breast, hippocampus, cardiac muscle, colon, and others.

**Public EST:** Blastx searches with the DNA sequence 028971 against the TREMBLP dataset identified a single homologous mouse EST (W85144) from the IMAGE Library, Soares  
15 mouse embryo NbME13.5-14.5. The blastx alignment of 028971 with W85144 and then with F55B12.3 revealed a change in reading frame in 028971 which probably is due to a sequencing error.

Blastn searches of the EMBL EST database with the 028971 DNA sequence revealed in addition to W85144, three human EST that align with the coding sequence of  
20 028971 and six EST that align with the 3' untranslated region of the 028971 sequence.

**Protein Motifs:** Two protein motifs were identified in F55B12.3 which are shared with yeast *cdc4*, mouse w85144 and human 028971. These are an F-box in the N-terminal domain and seven  $\beta$ -transducin repeats in the C-terminal domain.

25

### Discussion

The *sel-10* gene encodes a member of the  $\beta$ -transducin protein family. These are characterized by the presence of multiple WD40 repeats [Neer, E.J. *et al.*, *Nature* 371: 297-300 (1994)]. The repeats are 20-40 amino acids long and are bounded by gly-his (GH) and  
30 trp-asp (WD) residues. Solution of the three dimensional structure of  $\beta$ -transducin indicates that the WD40 repeats form the arms of a seven-bladed propeller like structure [Sondek, J. *et al.*, *Nature* 379: 369-74 (1996)]. Each blade is formed by four alternating pleats of beta-sheet with a pair of the conserved aspartic acid residues in the protein motif forming the

limits of one internal beta strand. WD40 repeats are found in over 27 different proteins which represent diverse functional classes [Neer, E.J. *et al.*, *Nature* 371: 297-300 (1994)]. These regulate cellular functions including cell division, cell fate determination, gene transcription, signal transduction, protein degradation, mRNA modification and vesicle fusion. This diversity in function has led to the hypothesis that  $\beta$ -transducin family members provide a common scaffolding upon which multiprotein complexes can be assembled.

The homology of *sel-10*, 28971 and W85144 to the yeast *cdc4* gene suggests a functional role in the ubiquitin-proteasome pathway for intracellular degradation of protein. Mutations of the yeast *cdc4* gene cause cell cycle arrest by blocking degradation of Sic1, an inhibitor of S-phase cyclin/cdk complexes [King, R.W. *et al.*, *Science* 274: 1652-9 (1996)]. Phosphorylation of Sic1 targets it for destruction through the ubiquitin-proteasome pathway. This pathway consists of three linked enzyme reactions that are catalyzed by multiprotein complexes [Ciechanover, A., *Cell* 79: 13-21 (1994); Ciechanover, A. and A.L. Schwartz, *FASEB J.* 8: 182-91 (1994)]. Initially, the C-terminal glycine of ubiquitin is activated by ATP to form a high energy thiol ester intermediate in a reaction catalyzed by the ubiquitin-activating enzyme, E1. Following activation, an E2 enzyme (ubiquitin conjugating enzyme) transfers ubiquitin from E1 to the protein target. In some cases, E2 acts alone. In others, it acts in concert with an E3 ubiquitin-ligating enzyme which binds the protein substrate and recruits an E2 to catalyze ubiquitination. E2 ubiquitin-conjugating enzymes comprise a fairly conserved gene family, while E3 enzymes are divergent in sequence [Ciechanover, A., *Cell* 79: 13-21 (1994); Ciechanover, A. and A.L. Schwartz, *FASEB J.* 8: 182-91 (1994)].

In yeast, mutation of the E2 ubiquitin-conjugating enzyme, *cdc34*, causes cell cycle arrest through failure to degrade the Sic1 inhibitor of the S-phase cyclin/cdk complex [King, R.W. *et al.*, *Science* 274: 1652-9 (1996)]. Sic1 normally is degraded as cells enter the G1-S phase transition, but in the absence of *cdc34*, Sic1 escapes degradation and its accumulation causes cell cycle arrest. Besides *cdc34*, *cdc4* is one of three other proteins required for the G1-S phase transition. The other two are *cdc53* and *Skp1*. As discussed above, *cdc4* contains two structural motifs, seven WD40 repeats (which suggests that the protein forms a beta-propeller) and a structural motif shared with cyclin F which is an interaction domain for *Skp1* [Bai, C. *et al.*, *Cell* 86: 263-74 (1996)]. Insect cell lysates containing *cdc53*, *cdc4* and *skp1* (and also ubiquitin, *cdc34* and E1) can transfer ubiquitin to Sic1 suggesting that one or more of these components functions as an E3 ubiquitin-ligating enzyme [King, R.W. *et al.*,

*Science* 274: 1652-9 (1996)]. Increased expression of either *cdc4* or *Skp1* partially rescues loss of the other.

In *C. elegans*, mutation of *sel-10* has no visible phenotype indicating that *sel-10* does not play a role in regulation of the cell-cycle. A closely related, *C. elegans*  $\beta$ -transducin family member, K10B2.6 may play that role as it clusters with the gene TRCP\_XEN from *Xenopus laevis* which rescues yeast cell cycle mutants arrested in late anaphase due to a failure to degrade cyclin B [Spevak, W. *et al.*, *Mol. Cell. Biol.* 13: 4953-66 (1993)]. If *sel-10* does encode a component of an E3-ubiquitin ligating enzyme, how might it suppress *sel-12* and enhance *lin-12* mutations? The simplest hypothesis is that *sel-10* regulates degradation of both proteins via the ubiquitin-proteasome pathway. Both *sel-12* and *lin-12* are transmembrane proteins. *Sel-12* crosses the membrane 8 times such that its N- and C-termini face the cytosol [Kim, T.W. *et al.*, *J. Biol. Chem.* 272: 11006-10 (1997)], while *lin-12* is a type 1 transmembrane protein (Greenwald, I. and G. Seydoux, *Nature* 346: 197-9 (1990)). Both are ubiquitinated, and in the case of human PS2, steady state levels increase in cells treated with an inhibitor of the proteasome, N-acetyl-L-leucinal-L-norleucinal and lactacystin (Li, X. and I. Greenwald, *Neuron*. 17: 1015-21 (1996)). Alternatively, *sel-10* may target for degradation of a negative regulator of presenilin function.

The genetic analysis and protein function suggested by homology to *cdc4* implies that drug inhibitors of human *sel-10* may increase steady state levels of human presenilins. This could potentiate activity of the presenilin pathway and provide a means for therapeutic intervention in Alzheimer's disease.

**Example 2: 5' RACE cloning of a human cDNA encoding *Sel-10*, an extragenic suppressor of presenilin mutations in *C. elegans***

**Materials and Methods**

Oligonucleotide primers for the amplification of the *sel-10* coding sequence from *C. elegans* cDNA were prepared based on the sequence of F55B12.3, identified in Example 1 as the coding sequence for *C. elegans sel-10*. The primers prepared were: 5'-CGGGATCCACCATGGATGATGGATCGATGACACC-3' (SEQ ID NO:11) and 5'-GGAATTCCTTAAGGGTATACAGCATCAAAGTCG-3' (SEQ ID NO:12). *C. elegans* mRNA was converted to cDNA using a BRL Superscript II Preamplification kit. The PCR product was digested with restriction enzymes *Bam*HI and *Eco*RI (LTI, Gaithersburg, MD)

and cloned into pcDNA3.1 (Invitrogen). Two isolates were sequenced (ABI, Perkin-Elmer Corp).

The sequence of Incyte clone 028971 (encoding a portion of the human homologue of *C. elegans* sel-10), was used to design four antisense oligonucleotide primers: 5'-  
 5 TCACTTCATGTCCACATCAAAGTCC-3' (SEQ ID NO:13), 5'-GGTAA-  
 TTACAAGTTCTTGTGAACTG (SEQ ID NO:14), 5'-CCCTGCAACGTGTGT-  
 AGACAGG-3' (SEQ ID NO:15), and 5'-CCAGTCTCTGCATTCCACACTTTG-3' (SEQ ID  
 NO:16) to amplify the missing 5' end of human sel-10. The Incyte LifeSeq "Electronic  
 Northern" analysis was used to identify tissues in which sel-10 was expressed. Two of these,  
 10 hippocampus and mammary gland, were chosen for 5' RACE cloning using a CloneTech  
 Marathon kit and prepared Marathon-ready cDNA from hippocampus and mammary gland.  
 PCR products were cloned into the TA vector pCR3.1 (Invitrogen), and isolates were  
 sequenced. An alternate 5' oligonucleotide primer was also designed based on Incyte clones  
 which have 5' ends that differ from the hippocampal sel-10 sequence:  
 15 5'-CTCAGACAGGTCAGGACATTTGG-3' (SEQ ID NO:17).

Blastn was used to search Incyte databases LifeSeq and LifeSeqFL. Gap  
 alignments and translations were performed with GCG programs (Wisconsin Sequence  
 Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park,  
 Madison Wisconsin).

20

### Results

*The coding sequence of the C. elegans sel-10:* The predicted coding sequence of the *C. elegans* sel-10, F55B12.3, had originally been determined at the Genome Sequencing  
 Center, Washington University, St. Louis, by using the computer program GeneFinder to  
 25 predict introns and exons in the genomic cosmid F55B12. The hypothetical cDNA sequence  
 was confirmed by amplifying this region from *C. elegans* cDNA, cloning, and sequencing it.

*The coding sequence of the human sel-10 gene homologue:* All of the 028971 antisense  
 oligonucleotides amplified a 5' RACE product from human hippocampal and mammary  
 30 cDNA. The longest PCR product from the hippocampal reactions was cloned and  
 sequenced. This PCR reaction was designed to generate products which end at the predicted  
 stop codon. Two isolates contained identical sequence which begins 880 bases before the  
 beginning of the 028971 sequence. This sequence was confirmed by comparison with



spanning Incyte cDNA clones. The Incyte clones that spanned the 5' end of the human *sel-10* homologue were not annotated as F55B12.3, as the homology in this region between the human and *C. elegans* genes is low, and as the overlap between these clones and the annotated clones happened to be too small for them to be clustered in the Incyte database or  
5 uncovered by our blasting the Incyte database with the 028971 sequence.

The predicted protein sequences of human *sel-10* have 47.6% identity and 56.7% similarity to *C. elegans sel-10*. The N-terminus of the human *sel-10* sequence begins with 4 in-frame methionines. In addition to the WD40 repeats described above, the human sequence also contains a region homologous to the CDC4 F-box for binding Skp1, as  
10 expected for a *sel-10* homologue.

*Different human sel-10 mRNAs expressed in mammary and hippocampal tissues:*

Several additional human *sel-10* ESTs which differ from the hippocampal sequence were identified. These are an exact match, which indicates that the alternative transcript is  
15 probably real. Comparison of these sequences with the human hippocampal *sel-10* sequence shows divergence prior to the 4th in-frame methionine and then exact sequence match thereafter. An oligonucleotide primer specific for the 5' end of this alternative transcript was found to amplify a product from mammary but not hippocampal cDNA. This indicates either that the human *sel-10* transcript undergoes differential splicing in a tissue-specific  
20 fashion or that the gene contains multiple, tissue specific promoters.

*Discussion*

5'RACE and PCR amplification were used to clone a full-length cDNA encoding the human homologue of the *C. elegans* gene, *sel-10*. Sequence analysis confirms the earlier  
25 prediction that *sel-10* is a member of the CDC4 family of proteins containing F-Box and WD40 Repeat domains. Two variants of the human *sel-10* cDNA were cloned from hippocampus and mammary gland which differed in 5' sequence preceding the apparent site of translation initiation. This implies that the gene may have two or more start sites for transcription initiation which are tissue-specific or that the pattern of exon splicing is tissue-  
30 specific.

**EXAMPLE 3: Expression Of Epitope-Tagged Sel-10 In Human Cells , and  
Perturbation Of Amyloid  $\beta$  Peptide Processing By Human Sel-10**

**Materials And Methods**

*Construction of Epitope-Tagged Sel-10: Subcloning, Cell Growth and Transfection:*

5 An EcoRI site was introduced in-frame into the human sel-10 cDNA using a polymerase chain reaction (PCR) primed with the oligonucleotides 237 (5'-GGAATTC-CATGAAAAGATTGGACCATGGTTCTG-3') (SEQ ID NO:18) and 206 (5'-GGA-ATTCCTCACTTCATGT-CACATCAAAGTCCAG-3') (SEQ ID NO:19). The resulting PCR product was cloned into the EcoRI site of the vector pCS2+MT. This fused a 5' 6-  
10 myc epitope tag in- frame to the fifth methionine of the hippocampal sel-10 cDNA, i.e., upstream of nucleotide 306 of the sequence given in SEQ ID NO:1. The nucleotide sequence of this construct, designated 6myc-N-sel-10, is given in SEQ ID NO: 20, while the amino acid sequence of the polypeptide encoded thereby is given in SEQ ID NO: 21. The hippocampal and mammary sel-10 cDNA diverge upstream of this methionine. A PS1  
15 cDNA with a 3'-FLAG tag (PS1-C-FLAG) was subcloned into the pcDNA3.1 vector. An APP cDNA containing the Swedish NL mutation and an attenuated ER retention sequence consisting of the addition of a di-lysyl motif to the C-terminus of APP695 (APP695NL-KK) was cloned into vector pIRES-EGFP (Mullan et al., Nat Genet 1992 Aug;1(5):345-7). HEK293 and IMR32 cells were grown to 80% confluence in DMEM with 10% FBS and  
20 transfected with the above cDNA. A total of 10 mg total DNA/6x10<sup>6</sup> cells was used for transfection with a single plasmid. For cotransfections of multiple plasmids, an equal amount of each plasmid was used for a total of 10 mg DNA using LipofectAmine (BRL).

In order to construct C-term V5 his tagged sel-10 and the C-term mychis tagged sel-10, the coding sequence of human hippocampal sel-10 was amplified using  
25 oligonucleotides primers containing a KpnI restriction site on the 5' primer: 5'-GGGTA-CCCCTCATTATTCCTCGAGTTCTTC-3' (SEQ ID NO:22) and an EcoRI site on the 3' primer: 5'-GGAATTCCTTCATGTCCACATCAAAGTCC-3' (SEQ ID NO:23), using the original human sel-10 RACE pcr product as template. The product was digested with both KpnI and EcoRI and cloned into either the vector pcDNA6/V5-His A or pcDNA3.1/Myc-  
30 His(+) A (Invitrogen). The nucleotide sequence of independent isolates was confirmed by dideoxy sequencing. The nucleotide sequence of the C-term V5 his tagged sel-10 is given in SEQ ID NO: 24, while the amino acid sequence of the polypeptide encoded thereby is given in SEQ ID NO: 25. The nucleotide sequence of independent isolates was confirmed

by dideoxy sequencing. The nucleotide sequence of the C-term mychis tagged sel-10 is given in SEQ ID NO: 26, while the amino acid sequence of the polypeptide encoded thereby is given in SEQ ID NO: 27.

*Clonal Selection of transformed cells by FACS:* Cell samples were analyzed on an EPICS Elite ESP flow cytometer (Coulter, Hialeah, FL) equipped with a 488 nm excitation line supplied by an air-cooled argon laser. EGFP emission was measured through a 525 nm band-pass filter and fluorescence intensity was displayed on a 4-decade log scale after gating on viable cells as determined by forward and right angle light scatter. Single green cells were separated into each well of one 96 well plate containing growth medium without G418. After a four day recovery period, G418 was added to the medium to a final concentration of 400 mg/ml. Wells with clones were expanded from the 96 well plate to a 24 well plate and then to a 6 well plate with the fastest growing colonies chosen for expansion at each passage.

*Immunofluorescence:* Cells grown on slides were fixed 48 hrs after transfection with 4% formaldehyde and 0.1% Triton X-100 in PBS for 30 min on ice and blocked with 10% Goat serum in PBS (blocking solution) 1 hr RT (i.e., 25°C), followed by incubation with mouse anti-myc (10 mg/ml) or rabbit anti-FLAG (0.5 mg/ml) antibody 4°C O/N and then fluorescein-labeled goat anti-mouse or anti-rabbit antibody (5mg/ml) in blocking solution 1 hr at 25°C.

*Western blotting:* Cell lysates were made 48 hrs after transfection by incubating 10<sup>5</sup> cells with 100 ml TENT (50 mM Tris-HCl pH 8.0, 2 mM EDTA, 150 mM NaCl, 1% Triton X-100, 1x protease inhibitor cocktail) 10 min on ice followed by centrifugation at 14,000 g. The supernatant was loaded on 4-12% NuPage gels (50 mg protein/lane) and electrophoresis and transfer were conducted using an Xcell II Mini-Cell system (Novex). The blot was blocked with 5% milk in PBS 1 hr RT and incubated with anti-myc or anti-FLAG antibody (described in "Immunofluorescence" above) 4°C O/N, then sheep anti-mouse or anti-rabbit antibody-HRP (0.1 mg/ml) 1 hr RT, followed by Supersignal (Pierce) detection.

*ELISA:* Cell culture supernatant or cell lysates (100 ml formic acid/10<sup>6</sup> cells) were assayed in the following double antibody sandwich ELISA. which is capable of detecting levels of A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> peptide in culture supernatant.

Human A $\beta$  1-40 or 1-42 was measured using monoclonal antibody (mAb) 6E10 (Senetek, St. Louis, MO) and biotinylated rabbit antiserum 162 or 164 (NYS Institute for

Basic Research, Staten Island, NY) in a double antibody sandwich ELISA. The capture antibody 6E10 is specific to an epitope present on the N-terminal amino acid residues 1-16 of hA $\beta$ . The conjugated detecting antibodies 162 and 164 are specific for hA $\beta$  1-40 and 1-42, respectively. The sandwich ELISA was performed according to the method of Pirttilä *et al.* (*Neurobiology of Aging* 18: 121-7 (1997)). Briefly, a Nunc Maxisorp 96 well immunoplate was coated with 100 $\mu$ l/well of mAb 6E10 (5 $\mu$ g/ml) diluted in 0.1M carbonate-bicarbonate buffer, pH 9.6 and incubated at 4°C overnight. After washing the plate 3x with 0.01M DPBS (Modified Dulbecco's Phosphate Buffered Saline (0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01 M potassium chloride, pH 7.4) from Pierce, Rockford, IL) containing 0.05% of Tween-20 (DPBST), the plate was blocked for 60 min with 200 $\mu$ l of 10% normal sheep serum (Sigma) in 0.01M DPBS to avoid non-specific binding. Human A $\beta$  1-40 or 1-42 standards 100 $\mu$ l/well (Bachem, Torrance, CA) diluted, from a 1mg/ml stock solution in DMSO, in non transfected conditioned cell medium was added after washing the plate, as well as 100 $\mu$ l/well of sample i.e. filtered conditioned medium of transfected cells. The plate was incubated for 2 hours at room temperature and 4°C overnight. The next day, after washing the plate, 100 $\mu$ l/well biotinylated rabbit antiserum 162 1:400 or 164 1:50 diluted in DPBST + 0.5% BSA was added and incubated at room temperature for 1hr 15 min. Following washes, 100 $\mu$ l/well neutravidin-horseradish peroxidase (Pierce, Rockford, IL) diluted 1:10,000 in DPBST was applied and incubated for 1 hr at room temperature. After the last washes 100 $\mu$ l/well of o-phenylenediamine dihydrochloride (Sigma Chemicals, St. Louis, MO) in 50mM citric acid/100mM sodium phosphate buffer (Sigma Chemicals, St. Louis, MO), pH 5.0, was added as substrate and the color development was monitored at 450nm in a kinetic microplate reader for 20 min. using Soft max Pro software.

## Results

**Transfection of HEK293 cells:** Transfection efficiency was monitored through the use of vectors that express green fluorescent protein (GFP) or by immunofluorescent detection of epitope-tagged sel-10 or PS1. An N-terminal 6-myc epitope was used to tag human sel-10 (6myc-N-sel-10), while PS1 was tagged with a C-terminal FLAG epitope (PS1-C-FLAG). APP695 was modified by inclusion of the Swedish NL mutation to increase A $\beta$  processing and an attenuated endoplasmic reticulum (ER) retention signal

consisting of a C-terminal di-lysine motif (APP695NL-KK). The di-lysine motif increases A $\beta$  processing about two fold. The APP695NL-KK construct was inserted into the first cistron of a bicistronic vector containing GFP (pIRES-EGFP, Invitrogen) to allow us to monitor transfection efficiency. Transfection efficiency in HEK293 cells was about 50% for transfections with a single plasmid DNA. For cotransfections with two plasmids, about 30-40% of the cells expressed both proteins as detected by double immunofluorescence.

Expression of recombinant protein in transfected HEK293 cells was confirmed by Western blot as illustrated for PS1-C-FLAG and 6myc-N-sel-10 (Fig 1A). In the case of cotransfections with three plasmids (PS1-C-FLAG + 6myc-N-sel-10 + APP), all three proteins were detected in the same cell lysate by Western blot (Figure 1B) using appropriate antibodies.

*Effect of 6myc-N-sel-10 and PS1-C-FLAG on A $\beta$  processing:* Cotransfection of APP695NL-KK with 6myc-N-sel-10 or PS1-C-FLAG into HEK293 cells increased the release of Ab1-40 and Ab1-42 peptide into the culture supernatant by 2- to 3-fold over transfections with just APP695NL-KK (Table 1). Cotransfection of APP695NL-KK with both 6myc-N-sel-10 and PS1-C-FLAG increased Ab release still further (i.e., 4- to 6-fold increase). In contrast, the ratio of Ab1-42/ (Ab1-40 + Ab1-42) released into the supernatant decreased about 50%. The subtle decrease in the ratio of Ab1-42/ (Ab1-40 + Ab1-42) reflects the larger increase in Ab 1-40 relative to Ab 1-42. Neither 6myc-N-sel-10 nor PS1-C-FLAG affected endogenous Ab production in HEK293 cells. Similar observations were also obtained in IMR32 cells (Table 2). However, IMR32 cells transfected less well than HEK293 cells, so the stimulation of APP695NL-KK processing by cotransfection with 6myc-N-sel-10 or PS1-C-FLAG was lower.

Levels of Ab 1-40 expressed in HEK293 cells transfected with APP695NL-KK were sufficient to measure Ab peptide in both the culture supernatant and cell pellet. Considerably more Ab 1-40 is detected in the HEK293 cell pellet than in the supernatant in cells transfected with just APP695NL-KK. Cotransfection with 6myc-N-sel-10 or PS1-C-FLAG proportionately decreased Ab 1-40 in the cell pellet and increased Ab in the culture supernatant. This implies that 6myc-N-sel-10 and PS1-C-FLAG alter processing or trafficking of APP such that proportionately more Ab is released from the cell.

*Effect of 6myc-N-sel-10 and PS1-C-FLAG expression on endogenous A $\beta$  processing:* The effect of 6myc-N-sel-10 on the processing of endogenous APP by human cells was assessed by creating stably transformed HEK293 cell lines expressing these

proteins. Two cell lines expressing 6myc-N-sel-10 were derived (sel-10/2 & sel-10/6) as well as a control cell line transformed with pcDNA3.1 vector DNA. Both 6myc-N-sel-10 cell lines expressed the protein as shown by Western blot analysis. Endogenous production of Ab 1-40 was increased in both 6myc-N-sel-10 cell lines in contrast to vector DNA transformed cells Table 3). In addition, stable expression of 6myc-N-sel-10 significantly increased Ab production after transfection with APP695NL-KK plasmid DNA (Table 3). Similar results were obtained with 6 stable cell lines expressing PS1-C-FLAG. All 6 cell lines showed significant elevation of endogenous A $\beta$  processing and all also showed enhanced processing of Ab after transfection with APP695NL-KK (Table 3). In addition, the increase of A $\beta$  processing seen with 6myc-N-sel-10 was also seen with sel-10 tagged at the C-terminus with either mychis or v5his (See Table 4). Both C-terminal and N-terminal tags resulted in an increase in A $\beta$  processing.

### Discussion

These data suggest that , when over expressed, 6myc-N-sel-10 as well as PS1-C-FLAG alter A $\beta$  processing in both transient and stable expression systems. A 6-myc epitope tag was used in these experiments to allow detection of sel-10 protein expression by Western blot analysis. If as its sequence homology to yeast CDC4 suggests, sel-10 is an E2-E3 ubiquitin ligase, it should be possible to identify the proteins it targets for ubiquitination. Since the presenilins are degraded via the ubiquitin-proteasome pathway, PS1 & PS2 are logical targets of sel-10 catalyzed ubiquitination [Kim *et al.*, *J. Biol. Chem.* 272:11006-11010 (1997)]. How sel-10 affects A $\beta$  processing is not understood at this point. In the future, it will be necessary to determine if sel-10 & PS1 increase A $\beta$  processing by altering production, processing, transport, or turn-over of APP, and whether the effect of PS1 is mediated or regulated by sel-10.

These experiments suggest that sel-10 is a potential drug target for decreasing Ab levels in the treatment of AD. They also show that *C. elegans* is an excellent model system in which to investigate presenilin biology in the context of AD. Thus, as is shown by cotransfection experiments, as well as in stable transformants, expression of 6myc-N-sel10 or PS1-C-FLAG increases A $\beta$  processing. An increase in A $\beta$  processing was seen in both HEK293 cells and IMR32 cells after cotransfection of 6myc-N-sel10 or PS1-C-FLAG with APP695NL-KK. In stable transformants of HEK293 cells expressing 6myc-Sel10 or PS1-

C-FLAG, an increase in endogenous A $\beta$  processing was observed, as well as an increase in A $\beta$  processing after transfection with APP695NL-KK. This suggests that inhibitors of either sel-10 and/or PS1, may decrease A $\beta$  processing, and could have therapeutic potential for Alzheimer's disease.

5           It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the invention.

10           The entire disclosure of all publications cited herein are hereby incorporated by reference.

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**Table 1.** Effect of 6myc-N-sel-10 and PS1-C-FLAG transient transfection on Ab levels in HEK293 cell supernatants.

Plasmids Transfected	Ab1-42 ng/ml	Ab1-40 ng/ml	Ab1-42/total Ab ng/ml
pcDNA3	81 ±20	231 ±50	0.26 ±0.05
6myc-N-sel-10	67 ±7	246 ±34	0.21 ±0.03
PS1-C-FLAG	75 ±18	227 ±45	0.25 ±0.03
PS1-C-FLAG + 6myc-N-sel-10	77 ±21	220 ±26	0.25 ±0.03
APP695NL-KK	141 ±27	896 ±103	0.14 ±0.02
APP695NL-KK + 6myc-N-sel-10	308 ±17	2576 ±190	0.11 ±0.00
APP695NL-KK + PS1-C-FLAG	364 ±39	3334 ±337	0.09 ±0.00
APP695NL-KK + PS1-C-FLAG + 6myc-N-sel-10	550 ±20	5897 ±388	0.09 ±0.00

5

**Table 2.** Effect of 6myc-N-sel-10 and PS1-C-FLAG transient transfection on Ab levels in IMR32 cell supernatants.

Plasmids Transfected	Ab1-42 ng/ml	Ab1-40 ng/ml	Ab1-42/total Ab ng/ml
pcDNA3	65 ±3	319 ±146	0.19 ±0.06
6myc-N-sel-10	63 ±0	246 ±53	0.21 ±0.04
PS1-C-FLAG	67 ±6	307 ±79	0.18 ±0.04
PS1-C-FLAG + 6myc-N-sel-10	67 ±6	302 ±94	0.20 ±0.08
APP695NL-KK	66 ±5	348 ±110	0.17 ±0.05
APP695NL-KK + 6myc-N-sel-10	75 ±18	448 ±141	0.15 ±0.03
APP695NL-KK + PS1-C-FLAG	63 ±26	466 ±72	0.12 ±0.02
APP695NL-KK + PS1-C-FLAG + 6myc-N-sel-10	81 ±26	565 ±179	0.12 ±0.01

10



## 6142.P CP

Table 3. Endogenous and exogenous Ab1-40 and Ab1-42 levels in supernatants from stable transformants of HEK293 cells.

Stable Line	GFP Transfection		APP695NL-KK Transfection	
	Ab1-40 ng/ml	Ab1-42 ng/ml	Ab1-40 ng/ml	Ab1-42 ng/ml
6myc-N-sel10/2	297 ± 29	109 ± 17	4877 ± 547	750 ± 32
6myc-N-sel10/6	168 ± 18	85 ± 11	8310 ± 308	1391 ± 19
PSI-C-FLAG/2	97 ± 6	68 ± 8	3348 ± 68	493 ± 21
PSI-C-FLAG/8	118 ± 11	85 ± 17	3516 ± 364	515 ± 36
PSI-C-FLAG/9	83 ± 20	67 ± 16	2369 ± 73	350 ± 12
PSI-C-FLAG/11	152 ± 17	68 ± 13	4771 ± 325	599 ± 25
PSI-C-FLAG/12	141 ± 12	50 ± 10	4095 ± 210	449 ± 21
PSI-C-FLAG/13	270 ± 139	61 ± 28	6983 ± 304	745 ± 41
pcDNA3/1	43 ± 13	75 ± 15	1960 ± 234	61 ± 6

5

Table 4. Sel-10 constructs with epitope tags at the N or C terminus increase Aβ 1-40 and Aβ 1-42.

construct	Aβ 1-40	Sel-10		Aβ 1-42	P-value	%increase	P-value
		ng/ml	%increase				
pcDNA	4240 ± 102			614 ± 10			
6myc-N-sel-10	7631 ± 465		80%	1136 ± 73	3.7 x 10 <sup>-6</sup>	46%	7.9 x 10 <sup>-6</sup>
sel-10-C-mychis	5485 ± 329		29%	795 ± 50	1.8 x 10 <sup>-4</sup>	29%	4.0 x 10 <sup>-4</sup>
sel-10-C-V5his	6210 ± 498		46%	906 ± 73	1.2 x 10 <sup>-4</sup>	48%	2.1 x 10 <sup>-4</sup>

***What is claimed is:***

1. An isolated nucleic acid molecule comprising a polynucleotide having a  
5 sequence at least 95% identical to a sequence selected from the group consisting of:
  - (a) a nucleotide sequence encoding a human sel-10 polypeptide having the complete amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, or as encoded by the cDNA clone contained in ATCC Deposit No.98978;
  - 10 (b) a nucleotide sequence encoding a human sel-10 polypeptide having the complete amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10, or as encoded by the cDNA clone contained in ATCC Deposit No. 98979; and
  - (c) a nucleotide sequence complementary to the nucleotide sequence of  
15 (a) or (b).
2. An isolated nucleic acid molecule comprising polynucleotide which hybridizes under stringent conditions to a polynucleotide having the nucleotide sequence in (a), (b), or (c) of claim 1.  
20
3. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(a) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:3.
- 25 4. The nucleic acid molecule of claim 3, wherein said polynucleotide molecule of 1(a) comprises the nucleotide sequence of residues 45-1928 of SEQ ID NO:1.
5. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(a) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID  
30 NO:4.
6. The nucleic acid molecule of claim 5, wherein said polynucleotide molecule of 1(a) comprises the nucleotide sequence of residues 150-1928 of SEQ ID NO:1.

7. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(a) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:5.

5 8. The nucleic acid molecule of claim 7, wherein said polynucleotide molecule of 1(a) comprises the nucleotide sequence of residues 267-1928 of SEQ ID NO:1.

9. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(a) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID  
10 NO:6.

10 10 The nucleic acid molecule of claim 9, wherein said polynucleotide molecule of 1(a) comprises the nucleotide sequence of residues 291-1928 of SEQ ID NO:1.

15 11. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(a) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:7.

12. The nucleic acid molecule of claim 11, wherein said polynucleotide  
20 molecule of 1(a) comprises the nucleotide sequence of residues 306-1928 of SEQ ID NO:1.

13. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(b) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:8.

25 14. The nucleic acid molecule of claim 13 wherein said polynucleotide molecule of 1(b) comprises the nucleotide sequence of residues 180-1949 of SEQ ID NO:2.

15. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(b)  
30 encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:9.

16. The nucleic acid molecule of claim 15, wherein said polynucleotide molecule of 1(b) comprises the nucleotide sequence of residues 270-1949 of SEQ ID NO:2.
- 5 17. The nucleic acid molecule of claim 1, wherein said polynucleotide of 1(b) encodes a human sel-10 polypeptide having the complete amino acid sequence of SEQ ID NO:10.
- 10 18. The nucleic acid molecule of claim 17, wherein said polynucleotide molecule of 1(b) comprises the nucleotide sequence of residues 327-1949 of SEQ ID NO:2.
19. A vector comprising the nucleic acid molecule of claim 1.
- 15 20. The vector of claim 19, wherein said nucleic acid molecule of claim 1 is operably linked to a promoter for the expression of a sel-10 polypeptide.
21. A host cell comprising the vector of claim 19.
- 20 22. The host cell of claim 21, wherein said host is a eukaryotic host.
23. A method of obtaining a sel-10 polypeptide comprising culturing the host cell of claim 22 and isolating said sel-10 polypeptide.
- 25 24. An isolated sel-10 polypeptide comprising
- (a) an amino acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, or as encoded by the cDNA clone contained in ATCC Deposit No. 98978;
- (b) an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10, or as encoded by the cDNA clone contained in ATCC Deposit No. 98979.
- 30

25. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:3.

26. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide  
5 comprises the amino acid sequence of SEQ ID NO:4.

27. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:5.

10 28. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:6.

29. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:7.

15 30. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:8.

31. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide  
20 comprises the amino acid sequence of SEQ ID NO:9.

32. The isolated sel-10 polypeptide of claim 24, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:10.

25 33. An isolated antibody that binds specifically to the sel-10 polypeptide of claim 24.

34. A cell line having altered A $\beta$  processing that expresses any of the sel-10  
isolated nucleic acid molecules of claim 1.  
30

35. The cell line of claim 34, wherein said A $\beta$  processing is increased.

35 36. The cell line of claim 34, wherein said A $\beta$  processing is decreased.

37. The cell line of claim 34, wherein said cell line is 6myc-N-sel10/2.

38. The cell line of claim 34, wherein said cell line is 6myc-N-sel10/6.

39. A method for the identification of an agent capable of altering the ratio of  $A\beta_{1-40}/A\beta_{1-40} + A\beta_{1-42}$  produced in any of the cell lines of claims 34, 37, and 38, comprising the steps of:

- 10 (a) obtaining a test culture and a control culture of said cell line;
- (b) contacting said test culture with a test agent;
- (c) measuring the levels of  $A\beta_{1-40}$  and  $A\beta_{1-42}$  produced by said test culture of step (b) and said control culture;
- (d) calculating the ratio of  $A\beta_{1-40}/A\beta_{1-40} + A\beta_{1-42}$  for said test culture
- 15 and said control culture from the levels of  $A\beta_{1-40}$  and  $A\beta_{1-42}$  measured in step (c); and
- (e) comparing the ratio of  $A\beta_{1-40}/A\beta_{1-40} + A\beta_{1-42}$  measured for said test culture and said control culture in step (d);

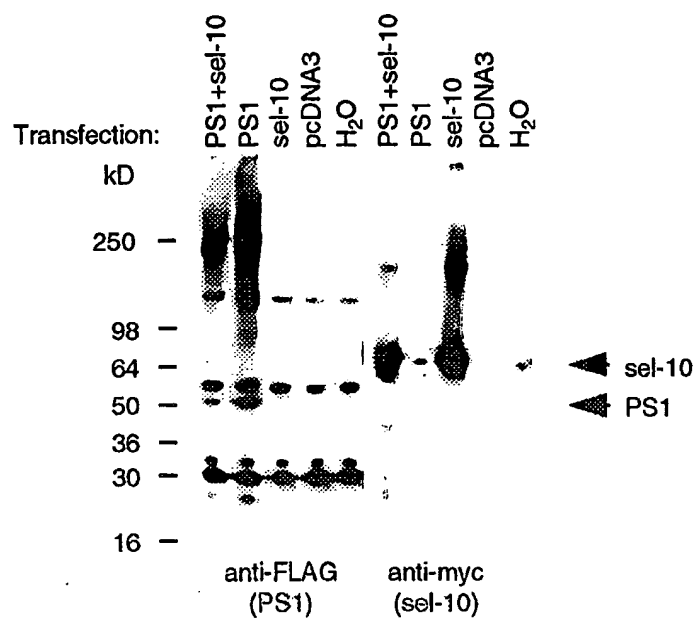
whereby a determination that the ratio of  $A\beta_{1-40}/A\beta_{1-40} + A\beta_{1-42}$  for said test culture is higher or lower than ratio of  $A\beta_{1-40}/A\beta_{1-40} + A\beta_{1-42}$  for said control culture indicates that

20 said test agent has altered the ratio of  $A\beta_{1-40}/A\beta_{1-40} + A\beta_{1-42}$ .

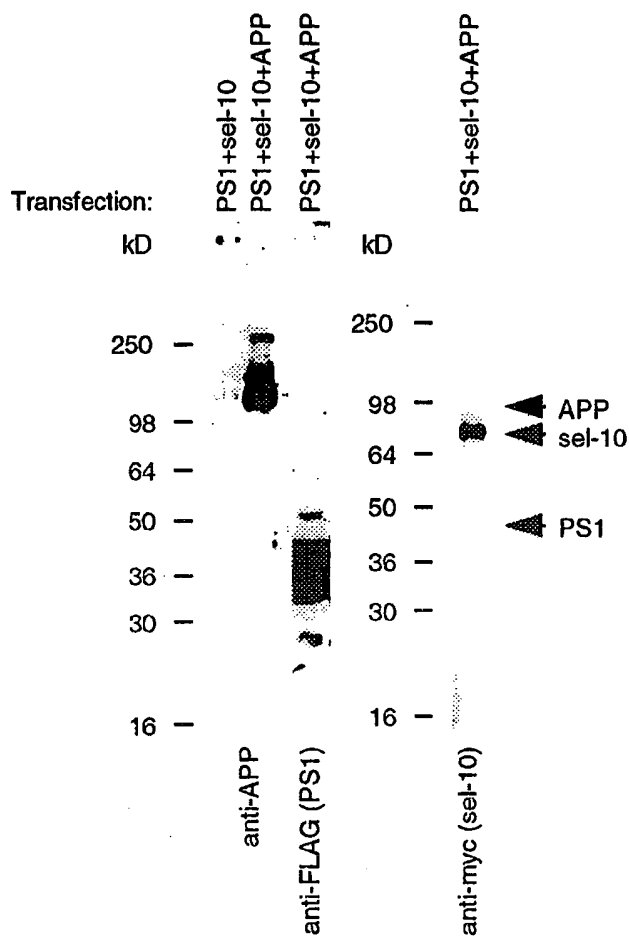
40. The method of claim 39, wherein said ratio of  $A\beta_{1-40}/A\beta_{1-40} + A\beta_{1-42}$  is increased by said test agent.

25 41. The method of claim 39, wherein said ratio of  $A\beta_{1-40}/A\beta_{1-40} + A\beta_{1-42}$  is decreased by said test agent.

30

**FIGURE 1A**

**FIGURE 1B**





## SEQUENCE LISTING

<110> Gurney, Mark E.

Li, Jinhe

Pauley, Adele M.

Pharmacia & Upjohn Company

<120> Human Sel-10 Polypeptides and Polynucleotides that  
Encode Them

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<140> 6142

<141> 1997-12-19

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cagattctgc tagcctgttc ttttaccact gacagctaga cacctagaaa ggaactgcaa 2340  
taatataaaa acaagtactg gttgactttc taattagaga gcactctgca caaaaagtca 2400  
tttttctgga gtggaaaagc ttaaaaaaat tactgtgaat tgtttttgta cagttatcat 2460  
gaaaagcttt tttttttatt ttttngccaa ccattgccaa tgtcaatcaa tcacagtatt 2520  
agcctctgtt aatctattta ctgttgcttc catatacatt cttcaatgca tatgttgctc 2580  
aaagggtggca agttgtcctg ggttctgtga gtctgagat ggatttaatt cttgatgctg 2640  
gtgctagaag taggtcttca aatatgggat tggtgtccca accctgtact gtactcccag 2700  
tggccaaact tatttatgct gctaaatgaa agaaagaaaa aagcaaatta ttttttttat 2760

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tttttttctg ctgtgacggt ttagtcccag actgaattcc aaatttgctc tagtttggtt 2820
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atggaagagt ttcagataat aaagagtga gtttgctgc aagtaaagaa ttgagagtgt 2940
gtgcaaagct tattttcttt tatctgggca aaaattaaaa cacattcctt ggaacagagc 3000
tattacttgc ctgttctgtg gagaaacttt tctttttgag ggctgtggtg aatggatgaa 3060
cgtacatcgt aaaactgaca aaatatttta aaaatatata aaacacaaaa ttaaaataaa 3120
gttgctgggtc agtcttagtg ttttacagta tttgggaaaa caactgttac agttttattg 3180
ctctgagtaa ctgacaaagc agaaactatt cagtttttgt agtaaaggcg tcacatgcaa 3240
acaaacaaaa tgaatgaaac agtcaaagtg tttgcctcat tctccaagag ccacaactca 3300
agctgaactg tgaaagtggg ttaacactgt atcctaggcg atcttttttc ctccttctgt 3360
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tatggtttga ttcacttaga aattttattt tcttataact taagtgaat aaaatgtgtt 3540
ttttcatgtt 3550

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&lt;210&gt; 2

&lt;211&gt; 3571

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 2

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ctcagcaggt caggacattt ggtaggggaa ggttgaaaga caaaagcagc aggccttggg 60
ttctcagcct tttaaaaact attattaaat atatatTTTTT aaaatttagt ggtagagct 120
tttagtaatg tgctgtatt acatgtagag agtattcgtc aaccaagagg agttttaaaa 180
tgtcaaaacc gggaaaacct actctaaacc atggcttggg tcctgttgat cttaaaagt 240
caaaagagcc tctaccacat caaacctga tgaagatatt tagcattagc atcattgccc 300
aaggcctccc tttttgtcga agacggatga aaagaaagt ggaccatggg tctgaggtcc 360
gctctttttc tttgggaaag aaaccatgca aagtctcaga atatacaagt accactgggc 420
ttgtaccatg ttcagcaaca ccaacaactt ttggggacct cagagcagcc aatggccaag 480
ggcaacaacg acgccgaatt acatctgtcc agccacctac aggcctccag gaatggctaa 540
aaatgtttca gagctggagt ggaccagaga aattgcttgc tttagatgaa ctattgata 600
gttgtgaacc aacacaagta aaacatatga tgcaagtgat agaaccacag tttcaacgag 660
acttcatttc attgctccct aaagagttgg cactctatgt gctttcattc ctggaaccca 720
aagacctgct acaagcagct cagacatgtc gctactggag aattttggct gaagacaacc 780
ttctctggag agagaaatgc aaagaagagg ggattgatga accattgcac atcaagagaa 840

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gaaaagtaat aaaaccaggt ttcatacaca gtccatggaa aagtgcatac atcagacagc 900  
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 atgatgatca tgtgatcaca tgcttacagt tttgtggtaa ccgaatagtt agtgggttctg 1020  
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 caaatttgct ctagtttgg tatggaaaa agactttttg cactgaaac ttgagccatc 2880  
 tgtgcctcta agaggctgag aatggaagag tttcagataa taaagagtga agtttgctg 2940

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caagtaaaga attgagagtg tgtgcaaagc ttattttctt ttatctgggc aaaaattaaa 3000
acacattcct tggaacagag ctattacttg cctgttctgt ggagaaactt ttctttttga 3060
gggctgtggt gaatggatga acgtacatcg taaaactgac aaaatatttt aaaaatatat 3120
aaaacacaaa attaaaataa agttgctggt cagtcttagt gttttacagt atttgggaaa 3180
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ttctccaaga gccacaactc aagctgaact gtgaaagtg tttaacactg tatectaggc 3360
gatctttttt cctccttctg tttatttttt tgnttgtttt atttatagtc tgatttaaaa 3420
caatcagatt caagttggtt aatttttagt atgtaacaac ctgacatgat ggaggaaaac 3480
aacctttaa gggattgtgt ctatggtttg attcacttag aaattttatt ttcttataac 3540
ttaagtgcaa taaaatgtgt tttttcatgt t                                     3571

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&lt;210&gt; 3

&lt;211&gt; 627

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 3

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Met Cys Val Pro Arg Ser Gly Leu Ile Leu Ser Cys Ile Cys Leu Tyr
1           5           10           15

```

```

Cys Gly Val Leu Leu Pro Val Leu Leu Pro Asn Leu Pro Phe Leu Thr
20           25           30

```

```

Cys Leu Ser Met Ser Thr Leu Glu Ser Val Thr Tyr Leu Pro Glu Lys
35           40           45

```

```

Gly Leu Tyr Cys Gln Arg Leu Pro Ser Ser Arg Thr His Gly Gly Thr
50           55           60

```

```

Glu Ser Leu Lys Gly Lys Asn Thr Glu Asn Met Gly Phe Tyr Gly Thr
65           70           75           80

```

```

Leu Lys Met Ile Phe Tyr Lys Met Lys Arg Lys Leu Asp His Gly Ser
85           90           95

```

Glu Val Arg Ser Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu  
 100 105 110

Tyr Thr Ser Thr Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr  
 115 120 125

Phe Gly Asp Leu Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg  
 130 135 140

Ile Thr Ser Val Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met  
 145 150 155 160

Phe Gln Ser Trp Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu  
 165 170 175

Ile Asp Ser Cys Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile  
 180 185 190

Glu Pro Gln Phe Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu  
 195 200 205

Ala Leu Tyr Val Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala  
 210 215 220

Ala Gln Thr Cys Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu  
 225 230 235 240

Trp Arg Glu Lys Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile  
 245 250 255

Lys Arg Arg Lys Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys  
 260 265 270

Ser Ala Tyr Ile Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly

275	280	285
Glu Leu Lys Ser Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile		
290	295	300
Thr Cys Leu Gln Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp		
305	310	315 320
Asn Thr Leu Lys Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr		
	325	330 335
Leu Val Gly His Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn		
	340	345 350
Ile Ile Ile Ser Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala		
355	360	365
Glu Thr Gly Glu Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val		
370	375	380
Arg Cys Met His Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp		
385	390	395 400
Ala Thr Leu Arg Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val		
	405	410 415
Leu Met Gly His Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg		
	420	425 430
Arg Val Val Ser Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro		
435	440	445
Glu Thr Glu Thr Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val		
450	455	460

Tyr Ser Leu Gln Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp  
465 470 475 480

Thr Ser Ile Arg Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr  
485 490 495

Leu Thr Gly His Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn  
500 505 510

Ile Leu Val Ser Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile  
515 520 525

Lys Thr Gly Gln Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln  
530 535 540

Ser Ala Val Thr Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser  
545 550 555 560

Ser Asp Asp Gly Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe  
565 570 575

Ile Arg Asn Leu Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val  
580 585 590

Trp Arg Ile Arg Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser  
595 600 605

Arg Asn Gly Thr Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val  
610 615 620

Asp Met Lys

625

<210> 4



&lt;211&gt; 592

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 4

Met Ser Thr Leu Glu Ser Val Thr Tyr Leu Pro Glu Lys Gly Leu Tyr  
 1 5 10 15

Cys Gln Arg Leu Pro Ser Ser Arg Thr His Gly Gly Thr Glu Ser Leu  
 20 25 30

Lys Gly Lys Asn Thr Glu Asn Met Gly Phe Tyr Gly Thr Leu Lys Met  
 35 40 45

Ile Phe Tyr Lys Met Lys Arg Lys Leu Asp His Gly Ser Glu Val Arg  
 50 55 60

Ser Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr Thr Ser  
 65 70 75 80

Thr Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr Phe Gly Asp  
 85 90 95

Leu Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile Thr Ser  
 100 105 110

Val Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met Phe Gln Ser  
 115 120 125

Trp Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile Asp Ser  
 130 135 140

Cys Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile Glu Pro Gln  
 145 150 155 160

Phe Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala Leu Tyr  
 165 170 175

Val Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala Gln Thr  
 180 185 190

Cys Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp Arg Glu  
 195 200 205

Lys Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile Lys Arg Arg  
 210 215 220

Lys Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys Ser Ala Tyr  
 225 230 235 240

Ile Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu Leu Lys  
 245 250 255

Ser Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile Thr Cys Leu  
 260 265 270

Gln Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp Asn Thr Leu  
 275 280 285

Lys Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr Leu Val Gly  
 290 295 300

His Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn Ile Ile Ile  
 305 310 315 320

Ser Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala Glu Thr Gly  
 325 330 335

Glu Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val Arg Cys Met  
 340 345 350

His Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp Ala Thr Leu  
 355 360 365

Arg Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val Leu Met Gly  
 370 375 380

His Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg Arg Val Val  
 385 390 395 400

Ser Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro Glu Thr Glu  
 405 410 415

Thr Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val Tyr Ser Leu  
 420 425 430

Gln Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp Thr Ser Ile  
 435 440 445

Arg Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr Leu Thr Gly  
 450 455 460

His Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn Ile Leu Val  
 465 470 475 480

Ser Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile Lys Thr Gly  
 485 490 495

Gln Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln Ser Ala Val  
 500 505 510

Thr Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser Ser Asp Asp  
 515 520 525

Gly Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile Arg Asn

530

535

540

Leu Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val Trp Arg Ile

545

550

555

560

Arg Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser Arg Asn Gly

565

570

575

Thr Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val Asp Met Lys

580

585

590

&lt;210&gt; 5

&lt;211&gt; 553

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 5

Met Gly Phe Tyr Gly Thr Leu Lys Met Ile Phe Tyr Lys Met Lys Arg

1

5

10

15

Lys Leu Asp His Gly Ser Glu Val Arg Ser Phe Ser Leu Gly Lys Lys

20

25

30

Pro Cys Lys Val Ser Glu Tyr Thr Ser Thr Thr Gly Leu Val Pro Cys

35

40

45

Ser Ala Thr Pro Thr Thr Phe Gly Asp Leu Arg Ala Ala Asn Gly Gln

50

55

60

Gly Gln Gln Arg Arg Arg Ile Thr Ser Val Gln Pro Pro Thr Gly Leu

65

70

75

80

Gln Glu Trp Leu Lys Met Phe Gln Ser Trp Ser Gly Pro Glu Lys Leu  
                                     85                                    90                                    95

Leu Ala Leu Asp Glu Leu Ile Asp Ser Cys Glu Pro Thr Gln Val Lys  
                                     100                                    105                                    110

His Met Met Gln Val Ile Glu Pro Gln Phe Gln Arg Asp Phe Ile Ser  
                                     115                                    120                                    125

Leu Leu Pro Lys Glu Leu Ala Leu Tyr Val Leu Ser Phe Leu Glu Pro  
                                     130                                    135                                    140

Lys Asp Leu Leu Gln Ala Ala Gln Thr Cys Arg Tyr Trp Arg Ile Leu  
 145                                    150                                    155                                    160

Ala Glu Asp Asn Leu Leu Trp Arg Glu Lys Cys Lys Glu Glu Gly Ile  
                                     165                                    170                                    175

Asp Glu Pro Leu His Ile Lys Arg Arg Lys Val Ile Lys Pro Gly Phe  
                                     180                                    185                                    190

Ile His Ser Pro Trp Lys Ser Ala Tyr Ile Arg Gln His Arg Ile Asp  
                                     195                                    200                                    205

Thr Asn Trp Arg Arg Gly Glu Leu Lys Ser Pro Lys Val Leu Lys Gly  
                                     210                                    215                                    220

His Asp Asp His Val Ile Thr Cys Leu Gln Phe Cys Gly Asn Arg Ile  
 225                                    230                                    235                                    240

Val Ser Gly Ser Asp Asp Asn Thr Leu Lys Val Trp Ser Ala Val Thr  
                                     245                                    250                                    255

Gly Lys Cys Leu Arg Thr Leu Val Gly His Thr Gly Gly Val Trp Ser

260 265 270  
Ser Gln Met Arg Asp Asn Ile Ile Ile Ser Gly Ser Thr Asp Arg Thr  
275 280 285  
Leu Lys Val Trp Asn Ala Glu Thr Gly Glu Cys Ile His Thr Leu Tyr  
290 295 300  
Gly His Thr Ser Thr Val Arg Cys Met His Leu His Glu Lys Arg Val  
305 310 315 320  
Val Ser Gly Ser Arg Asp Ala Thr Leu Arg Val Trp Asp Ile Glu Thr  
325 330 335  
Gly Gln Cys Leu His Val Leu Met Gly His Val Ala Ala Val Arg Cys  
340 345 350  
Val Gln Tyr Asp Gly Arg Arg Val Val Ser Gly Ala Tyr Asp Phe Met  
355 360 365  
Val Lys Val Trp Asp Pro Glu Thr Glu Thr Cys Leu His Thr Leu Gln  
370 375 380  
Gly His Thr Asn Arg Val Tyr Ser Leu Gln Phe Asp Gly Ile His Val  
385 390 395 400  
Val Ser Gly Ser Leu Asp Thr Ser Ile Arg Val Trp Asp Val Glu Thr  
405 410 415  
Gly Asn Cys Ile His Thr Leu Thr Gly His Gln Ser Leu Thr Ser Gly  
420 425 430  
Met Glu Leu Lys Asp Asn Ile Leu Val Ser Gly Asn Ala Asp Ser Thr  
435 440 445

Val Lys Ile Trp Asp Ile Lys Thr Gly Gln Cys Leu Gln Thr Leu Gln

450

455

460

Gly Pro Asn Lys His Gln Ser Ala Val Thr Cys Leu Gln Phe Asn Lys

465

470

475

480

Asn Phe Val Ile Thr Ser Ser Asp Asp Gly Thr Val Lys Leu Trp Asp

485

490

495

Leu Lys Thr Gly Glu Phe Ile Arg Asn Leu Val Thr Leu Glu Ser Gly

500

505

510

Gly Ser Gly Gly Val Val Trp Arg Ile Arg Ala Ser Asn Thr Lys Leu

515

520

525

Val Cys Ala Val Gly Ser Arg Asn Gly Thr Glu Glu Thr Lys Leu Leu

530

535

540

Val Leu Asp Phe Asp Val Asp Met Lys

545

550

&lt;210&gt; 6

&lt;211&gt; 545

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 6

Met Ile Phe Tyr Lys Met Lys Arg Lys Leu Asp His Gly Ser Glu Val

1

5

10

15

Arg Ser Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr Thr

20

25

30

Ser Thr Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr Phe Gly

35 40 45

Asp Leu Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile Thr  
50 55 60

Ser Val Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met Phe Gln  
65 70 75 80

Ser Trp Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile Asp  
85 90 95

Ser Cys Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile Glu Pro  
100 105 110

Gln Phe Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala Leu  
115 120 125

Tyr Val Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala Gln  
130 135 140

Thr Cys Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp Arg  
145 150 155 160

Glu Lys Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile Lys Arg  
165 170 175

Arg Lys Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys Ser Ala  
180 185 190

Tyr Ile Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu Leu  
195 200 205

Lys Ser Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile Thr Cys  
210 215 220



Leu Gln Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp Asn Thr  
 225 230 235 240

Leu Lys Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr Leu Val  
 245 250 255

Gly His Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn Ile Ile  
 260 265 270

Ile Ser Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala Glu Thr  
 275 280 285

Gly Glu Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val Arg Cys  
 290 295 300

Met His Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp Ala Thr  
 305 310 315 320

Leu Arg Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val Leu Met  
 325 330 335

Gly His Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg Arg Val  
 340 345 350

Val Ser Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro Glu Thr  
 355 360 365

Glu Thr Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val Tyr Ser  
 370 375 380

Leu Gln Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp Thr Ser  
 385 390 395 400

Ile Arg Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr Leu Thr  
 405 410 415

Gly His Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn Ile Leu  
420 425 430

Val Ser Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile Lys Thr  
435 440 445

Gly Gln Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln Ser Ala  
450 455 460

Val Thr Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser Ser Asp  
465 470 475 480

Asp Gly Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile Arg  
485 490 495

Asn Leu Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val Trp Arg  
500 505 510

Ile Arg Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser Arg Asn  
515 520 525

Gly Thr Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val Asp Met  
530 535 540

Lys

545

<210> 7

<211> 540

<212> PRT

<213> Homo sapiens

<400> 7

Met Lys Arg Lys Leu Asp His Gly Ser Glu Val Arg Ser Phe Ser Leu  
 1 5 10 15  
 Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr Thr Ser Thr Thr Gly Leu  
 20 25 30  
 Val Pro Cys Ser Ala Thr Pro Thr Thr Phe Gly Asp Leu Arg Ala Ala  
 35 40 45  
 Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile Thr Ser Val Gln Pro Pro  
 50 55 60  
 Thr Gly Leu Gln Glu Trp Leu Lys Met Phe Gln Ser Trp Ser Gly Pro  
 65 70 75 80  
 Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile Asp Ser Cys Glu Pro Thr  
 85 90 95  
 Gln Val Lys His Met Met Gln Val Ile Glu Pro Gln Phe Gln Arg Asp  
 100 105 110  
 Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala Leu Tyr Val Leu Ser Phe  
 115 120 125  
 Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala Gln Thr Cys Arg Tyr Trp  
 130 135 140  
 Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp Arg Glu Lys Cys Lys Glu  
 145 150 155 160  
 Glu Gly Ile Asp Glu Pro Leu His Ile Lys Arg Arg Lys Val Ile Lys  
 165 170 175  
 Pro Gly Phe Ile His Ser Pro Trp Lys Ser Ala Tyr Ile Arg Gln His  
 180 185 190

Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu Leu Lys Ser Pro Lys Val  
 195 200 205

Leu Lys Gly His Asp Asp His Val Ile Thr Cys Leu Gln Phe Cys Gly  
 210 215 220

Asn Arg Ile Val Ser Gly Ser Asp Asp Asn Thr Leu Lys Val Trp Ser  
 225 230 235 240

Ala Val Thr Gly Lys Cys Leu Arg Thr Leu Val Gly His Thr Gly Gly  
 245 250 255

Val Trp Ser Ser Gln Met Arg Asp Asn Ile Ile Ile Ser Gly Ser Thr  
 260 265 270

Asp Arg Thr Leu Lys Val Trp Asn Ala Glu Thr Gly Glu Cys Ile His  
 275 280 285

Thr Leu Tyr Gly His Thr Ser Thr Val Arg Cys Met His Leu His Glu  
 290 295 300

Lys Arg Val Val Ser Gly Ser Arg Asp Ala Thr Leu Arg Val Trp Asp  
 305 310 315 320

Ile Glu Thr Gly Gln Cys Leu His Val Leu Met Gly His Val Ala Ala  
 325 330 335

Val Arg Cys Val Gln Tyr Asp Gly Arg Arg Val Val Ser Gly Ala Tyr  
 340 345 350

Asp Phe Met Val Lys Val Trp Asp Pro Glu Thr Glu Thr Cys Leu His  
 355 360 365

Thr Leu Gln Gly His Thr Asn Arg Val Tyr Ser Leu Gln Phe Asp Gly

370	375	380	
Ile His Val Val Ser Gly Ser Leu Asp Thr Ser Ile Arg Val Trp Asp			
385	390	395	400
Val Glu Thr Gly Asn Cys Ile His Thr Leu Thr Gly His Gln Ser Leu			
	405	410	415
Thr Ser Gly Met Glu Leu Lys Asp Asn Ile Leu Val Ser Gly Asn Ala			
	420	425	430
Asp Ser Thr Val Lys Ile Trp Asp Ile Lys Thr Gly Gln Cys Leu Gln			
	435	440	445
Thr Leu Gln Gly Pro Asn Lys His Gln Ser Ala Val Thr Cys Leu Gln			
	450	455	460
Phe Asn Lys Asn Phe Val Ile Thr Ser Ser Asp Asp Gly Thr Val Lys			
465	470	475	480
Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile Arg Asn Leu Val Thr Leu			
	485	490	495
Glu Ser Gly Gly Ser Gly Gly Val Val Trp Arg Ile Arg Ala Ser Asn			
	500	505	510
Thr Lys Leu Val Cys Ala Val Gly Ser Arg Asn Gly Thr Glu Glu Thr			
	515	520	525
Lys Leu Leu Val Leu Asp Phe Asp Val Asp Met Lys			
	530	535	540

&lt;210&gt; 8

&lt;211&gt; 589

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 8

Met Ser Lys Pro Gly Lys Pro Thr Leu Asn His Gly Leu Val Pro Val

1 5 10 15

Asp Leu Lys Ser Ala Lys Glu Pro Leu Pro His Gln Thr Val Met Lys

20 25 30

Ile Phe Ser Ile Ser Ile Ile Ala Gln Gly Leu Pro Phe Cys Arg Arg

35 40 45

Arg Met Lys Arg Lys Leu Asp His Gly Ser Glu Val Arg Ser Phe Ser

50 55 60

Leu Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr Thr Ser Thr Thr Gly

65 70 75 80

Leu Val Pro Cys Ser Ala Thr Pro Thr Thr Phe Gly Asp Leu Arg Ala

85 90 95

Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile Thr Ser Val Gln Pro

100 105 110

Pro Thr Gly Leu Gln Glu Trp Leu Lys Met Phe Gln Ser Trp Ser Gly

115 120 125

Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile Asp Ser Cys Glu Pro

130 135 140

Thr Gln Val Lys His Met Met Gln Val Ile Glu Pro Gln Phe Gln Arg

145 150 155 160

Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala Leu Tyr Val Leu Ser

	165	170	175
Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala Gln Thr Cys Arg Tyr			
	180	185	190
Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp Arg Glu Lys Cys Lys			
	195	200	205
Glu Glu Gly Ile Asp Glu Pro Leu His Ile Lys Arg Arg Lys Val Ile			
	210	215	220
Lys Pro Gly Phe Ile His Ser Pro Trp Lys Ser Ala Tyr Ile Arg Gln			
	225	230	235
His Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu Leu Lys Ser Pro Lys			
	245	250	255
Val Leu Lys Gly His Asp Asp His Val Ile Thr Cys Leu Gln Phe Cys			
	260	265	270
Gly Asn Arg Ile Val Ser Gly Ser Asp Asp Asn Thr Leu Lys Val Trp			
	275	280	285
Ser Ala Val Thr Gly Lys Cys Leu Arg Thr Leu Val Gly His Thr Gly			
	290	295	300
Gly Val Trp Ser Ser Gln Met Arg Asp Asn Ile Ile Ile Ser Gly Ser			
	305	310	315
Thr Asp Arg Thr Leu Lys Val Trp Asn Ala Glu Thr Gly Glu Cys Ile			
	325	330	335
His Thr Leu Tyr Gly His Thr Ser Thr Val Arg Cys Met His Leu His			
	340	345	350

Glu Lys Arg Val Val Ser Gly Ser Arg Asp Ala Thr Leu Arg Val Trp  
 355 360 365

Asp Ile Glu Thr Gly Gln Cys Leu His Val Leu Met Gly His Val Ala  
 370 375 380

Ala Val Arg Cys Val Gln Tyr Asp Gly Arg Arg Val Val Ser Gly Ala  
 385 390 395 400

Tyr Asp Phe Met Val Lys Val Trp Asp Pro Glu Thr Glu Thr Cys Leu  
 405 410 415

His Thr Leu Gln Gly His Thr Asn Arg Val Tyr Ser Leu Gln Phe Asp  
 420 425 430

Gly Ile His Val Val Ser Gly Ser Leu Asp Thr Ser Ile Arg Val Trp  
 435 440 445

Asp Val Glu Thr Gly Asn Cys Ile His Thr Leu Thr Gly His Gln Ser  
 450 455 460

Leu Thr Ser Gly Met Glu Leu Lys Asp Asn Ile Leu Val Ser Gly Asn  
 465 470 475 480

Ala Asp Ser Thr Val Lys Ile Trp Asp Ile Lys Thr Gly Gln Cys Leu  
 485 490 495

Gln Thr Leu Gln Gly Pro Asn Lys His Gln Ser Ala Val Thr Cys Leu  
 500 505 510

Gln Phe Asn Lys Asn Phe Val Ile Thr Ser Ser Asp Asp Gly Thr Val  
 515 520 525

Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile Arg Asn Leu Val Thr  
 530 535 540



Leu Glu Ser Gly Gly Ser Gly Gly Val Val Trp Arg Ile Arg Ala Ser  
 545 550 555 560

Asn Thr Lys Leu Val Cys Ala Val Gly Ser Arg Asn Gly Thr Glu Glu  
 565 570 575

Thr Lys Leu Leu Val Leu Asp Phe Asp Val Asp Met Lys  
 580 585

<210> 9

<211> 559

<212> PRT

<213> Homo sapiens

<400> 9

Met Lys Ile Phe Ser Ile Ser Ile Ile Ala Gln Gly Leu Pro Phe Cys  
 1 5 10 15

Arg Arg Arg Met Lys Arg Lys Leu Asp His Gly Ser Glu Val Arg Ser  
 20 25 30

Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr Thr Ser Thr  
 35 40 45

Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr Phe Gly Asp Leu  
 50 55 60

Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile Thr Ser Val  
 65 70 75 80

Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met Phe Gln Ser Trp  
 85 90 95

Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile Asp Ser Cys  
 100 105 110

Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile Glu Pro Gln Phe  
 115 120 125

Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala Leu Tyr Val  
 130 135 140

Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala Gln Thr Cys  
 145 150 155 160

Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp Arg Glu Lys  
 165 170 175

Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile Lys Arg Arg Lys  
 180 185 190

Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys Ser Ala Tyr Ile  
 195 200 205

Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu Leu Lys Ser  
 210 215 220

Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile Thr Cys Leu Gln  
 225 230 235 240

Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp Asn Thr Leu Lys  
 245 250 255

Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr Leu Val Gly His  
 260 265 270

Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn Ile Ile Ile Ser  
 275 280 285

Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala Glu Thr Gly Glu  
 290 295 300

Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val Arg Cys Met His  
 305 310 315 320

Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp Ala Thr Leu Arg  
 325 330 335

Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val Leu Met Gly His  
 340 345 350

Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg Arg Val Val Ser  
 355 360 365

Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro Glu Thr Glu Thr  
 370 375 380

Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val Tyr Ser Leu Gln  
 385 390 395 400

Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp Thr Ser Ile Arg  
 405 410 415

Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr Leu Thr Gly His  
 420 425 430

Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn Ile Leu Val Ser  
 435 440 445

Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile Lys Thr Gly Gln  
 450 455 460

Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln Ser Ala Val Thr

465                      470                      475                      480  
 Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser Ser Asp Asp Gly  
                     485                      490                      495  
 Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile Arg Asn Leu  
                     500                      505                      510  
 Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val Trp Arg Ile Arg  
                     515                      520                      525  
 Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser Arg Asn Gly Thr  
                     530                      535                      540  
 Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val Asp Met Lys  
 545                      550                      555  
  
 <210> 10  
 <211> 540  
 <212> PRT  
 <213> Homo sapiens  
  
 <400> 10  
 Met Lys Arg Lys Leu Asp His Gly Ser Glu Val Arg Ser Phe Ser Leu  
     1                      5                      10                      15  
 Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr Thr Ser Thr Thr Gly Leu  
                     20                      25                      30  
 Val Pro Cys Ser Ala Thr Pro Thr Thr Phe Gly Asp Leu Arg Ala Ala  
                     35                      40                      45  
 Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile Thr Ser Val Gln Pro Pro  
                     50                      55                      60

Thr Gly Leu Gln Glu Trp Leu Lys Met Phe Gln Ser Trp Ser Gly Pro  
 65 70 75 80

Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile Asp Ser Cys Glu Pro Thr  
 85 90 95

Gln Val Lys His Met Met Gln Val Ile Glu Pro Gln Phe Gln Arg Asp  
 100 105 110

Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala Leu Tyr Val Leu Ser Phe  
 115 120 125

Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala Gln Thr Cys Arg Tyr Trp  
 130 135 140

Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp Arg Glu Lys Cys Lys Glu  
 145 150 155 160

Glu Gly Ile Asp Glu Pro Leu His Ile Lys Arg Arg Lys Val Ile Lys  
 165 170 175

Pro Gly Phe Ile His Ser Pro Trp Lys Ser Ala Tyr Ile Arg Gln His  
 180 185 190

Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu Leu Lys Ser Pro Lys Val  
 195 200 205

Leu Lys Gly His Asp Asp His Val Ile Thr Cys Leu Gln Phe Cys Gly  
 210 215 220

Asn Arg Ile Val Ser Gly Ser Asp Asp Asn Thr Leu Lys Val Trp Ser  
 225 230 235 240

Ala Val Thr Gly Lys Cys Leu Arg Thr Leu Val Gly His Thr Gly Gly

245 250 255  
Val Trp Ser Ser Gln Met Arg Asp Asn Ile Ile Ile Ser Gly Ser Thr  
260 265 270  
Asp Arg Thr Leu Lys Val Trp Asn Ala Glu Thr Gly Glu Cys Ile His  
275 280 285  
Thr Leu Tyr Gly His Thr Ser Thr Val Arg Cys Met His Leu His Glu  
290 295 300  
Lys Arg Val Val Ser Gly Ser Arg Asp Ala Thr Leu Arg Val Trp Asp  
305 310 315 320  
Ile Glu Thr Gly Gln Cys Leu His Val Leu Met Gly His Val Ala Ala  
325 330 335  
Val Arg Cys Val Gln Tyr Asp Gly Arg Arg Val Val Ser Gly Ala Tyr  
340 345 350  
Asp Phe Met Val Lys Val Trp Asp Pro Glu Thr Glu Thr Cys Leu His  
355 360 365  
Thr Leu Gln Gly His Thr Asn Arg Val Tyr Ser Leu Gln Phe Asp Gly  
370 375 380  
Ile His Val Val Ser Gly Ser Leu Asp Thr Ser Ile Arg Val Trp Asp  
385 390 395 400  
Val Glu Thr Gly Asn Cys Ile His Thr Leu Thr Gly His Gln Ser Leu  
405 410 415  
Thr Ser Gly Met Glu Leu Lys Asp Asn Ile Leu Val Ser Gly Asn Ala  
420 425 430

Asp Ser Thr Val Lys Ile Trp Asp Ile Lys Thr Gly Gln Cys Leu Gln

435

440

445

Thr Leu Gln Gly Pro Asn Lys His Gln Ser Ala Val Thr Cys Leu Gln

450

455

460

Phe Asn Lys Asn Phe Val Ile Thr Ser Ser Asp Asp Gly Thr Val Lys

465

470

475

480

Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile Arg Asn Leu Val Thr Leu

485

490

495

Glu Ser Gly Gly Ser Gly Gly Val Val Trp Arg Ile Arg Ala Ser Asn

500

505

510

Thr Lys Leu Val Cys Ala Val Gly Ser Arg Asn Gly Thr Glu Glu Thr

515

520

525

Lys Leu Leu Val Leu Asp Phe Asp Val Asp Met Lys

530

535

540

<210> 11

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 11

cgggatccac catggatgat ggatcgatga cacc

34

<210> 12

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 12

ggaattcctt aagggtatac agcatcaaag tcg

33

<210> 13

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 13

tcacttcacg tccacatcaa agtcc

25

<210> 14

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 14

ggtaattaca agttcttggt gaactg

26



<210> 15

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 15

ccctgcaacg tgtgtagaca gg

22

<210> 16

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 16

ccagtctctg cattccacac ttg

24

<210> 17

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 17

ctcagacagg tcaggacatt tgg

23

<210> 18

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 18

ggaattccat gaaaagattg gaccatgggtt ctg

33

<210> 19

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 19

ggaattcctc acttcatgtc acatcaaagt ccag

34

<210> 20

<211> 1881

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: 6 myc tagged

homo sapiens

<400> 20

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atggagcaaa agctcatttc tgaagaggac ttgaatgaaa tggagcaaaa gctcatttct 60
gaagaggact tgaatgaaat ggagcaaaaag ctcatattctg aagaggactt gaatgaaatg 120
gagcaaaaagc tcattttctga agaggacttg aatgaaatgg agcaaaaagct cattttctgaa 180
gaggacttga atgaaatgga gagcttgggc gacctcacca tggagcaaaa gctcatttct 240
gaagaggact tgaattccat gaaaagaaaag ttggaccatg gttctgaggt ccgctctttt 300
tctttgggaa agaaaccatg caaagtctca gaatatacaa gtaccactgg gcttgtacca 360
tgttcagcaa caccaacaac ttttggggac ctgagagcag ccaatggcca agggcaacaa 420
cgacgccgaa ttacatctgt ccagccacct acaggcctcc aggaatggct aaaaatgttt 480
cagagctgga gtggaccaga gaaattgctt gcttttagatg aactcattga tagttgtgaa 540
ccaacacaag taaaacatat gatgcaagtg atagaacccc agtttcaacg agacttcatt 600
tcattgctcc ctaaagagtt ggcaactctat gtgctttcat tcctggaacc caaagacctg 660
ctacaagcag ctgagacatg tcgctactgg agaatttttg ctgaagacaa ccttctctgg 720
agagagaaat gcaaagaaga ggggattgat gaaccattgc acatcaagag aagaaaagta 780
ataaaaccag gtttcataca cagtccatgg aaaagtgcac acatcagaca gcacagaatt 840
gatactaact ggagggcagg agaactcaaa tctcctaagg tgctgaaagg acatgatgat 900
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ggtggagtat ggtcatcaca aatgaggggac aacatcatca ttagtggatc tacagatcgg 1080
acactcaaag tgtggaatgc agagactgga gaatgtatac acaccttata tgggcatact 1140
tccactgtgc gttgtatgca tcttcatgaa aaaagagttg ttagcggttc tcgagatgcc 1200
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tcgttaacaa gtggaatgga actcaaagac aatattcttg tctctgggaa tgcagattct 1560
acagttaaaa tctgggatat caaaacagga cagtgtttac aaacattgca aggtcccaac 1620
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gatgatggaa ctgtaaaact atgggacttg aaaacgggtg aatttattcg aaacctagtc 1740
acattggaga gtggggggag tgggggagtt gtgtggcgga tcagagcctc aaacacaaag 1800
ctggtgtgtg cagttgggag tcggaatggg actgaagaaa ccaagctgct ggtgctggac 1860
tttgatgtgg acatgaagtg a

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1881

&lt;210&gt; 21

&lt;211&gt; 626

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: 6 myc tagged  
homo sapien

&lt;400&gt; 21

Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Glu Met Glu Gln

1                      5                      10                      15

Lys Leu Ile Ser Glu Glu Asp Leu Asn Glu Met Glu Gln Lys Leu Ile

20                      25                      30

Ser Glu Glu Asp Leu Asn Glu Met Glu Gln Lys Leu Ile Ser Glu Glu

35                      40                      45

Asp Leu Asn Glu Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn

50                      55                      60

Glu Met Glu Ser Leu Gly Asp Leu Thr Met Glu Gln Lys Leu Ile Ser

65                      70                      75                      80

Glu Glu Asp Leu Asn Ser Met Lys Arg Lys Leu Asp His Gly Ser Glu

85                      90                      95

Val Arg Ser Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu Tyr

100                      105                      110

Thr Ser Thr Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr Phe

115                      120                      125

Gly Asp Leu Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg Ile

130                      135                      140

Thr Ser Val Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met Phe			
145	150	155	160
Gln Ser Trp Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu Ile			
	165	170	175
Asp Ser Cys Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile Glu			
	180	185	190
Pro Gln Phe Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu Ala			
	195	200	205
Leu Tyr Val Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala Ala			
	210	215	220
Gln Thr Cys Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu Trp			
225	230	235	240
Arg Glu Lys Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile Lys			
	245	250	255
Arg Arg Lys Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys Ser			
	260	265	270
Ala Tyr Ile Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly Glu			
	275	280	285
Leu Lys Ser Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile Thr			
	290	295	300
Cys Leu Gln Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp Asn			
305	310	315	320
Thr Leu Lys Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr Leu			
	325	330	335

Val Gly His Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn Ile  
340 345 350

Ile Ile Ser Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala Glu  
355 360 365

Thr Gly Glu Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val Arg  
370 375 380

Cys Met His Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp Ala  
385 390 395 400

Thr Leu Arg Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val Leu  
405 410 415

Met Gly His Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg Arg  
420 425 430

Val Val Ser Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro Glu  
435 440 445

Thr Glu Thr Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val Tyr  
450 455 460

Ser Leu Gln Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp Thr  
465 470 475 480

Ser Ile Arg Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr Leu  
485 490 495

Thr Gly His Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn Ile  
500 505 510

Leu Val Ser Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile Lys

515	520	525
Thr Gly Gln Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln Ser		
530	535	540
Ala Val Thr Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser Ser		
545	550	555
		560
Asp Asp Gly Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe Ile		
565	570	575
Arg Asn Leu Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val Trp		
580	585	590
Arg Ile Arg Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser Arg		
595	600	605
Asn Gly Thr Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val Asp		
610	615	620
Met Lys		
625		

&lt;210&gt; 22

&lt;211&gt; 31

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence:

Oligonucleotide primer

&lt;400&gt; 22

gggtaccct cattattccc tcgagttctt c

31

<210> 23

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

Oligonucleotide primer

<400> 23

ggaattcctt catgtccaca tcaaagtcc

29

<210> 24

<211> 2010

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: V5HIS tagged

homo sapien

<400> 24

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 ttgccggttc tgctccctaa tcttcctttt ctgacgtgcc tgagcatgtc cacattagaa 120  
 tctgtgacat acctacctga aaaagggtta tattgtcaga gactgccaaag cagccggaca 180  
 cacgggggca cagaatcact gaaggggaaa aatacagaaa atatgggttt ctacggcaca 240  
 ttaaaaatga ttttttacia aatgaaaaga aagttggacc atggttctga ggtccgctct 300  
 ttttcttttg gaaagaaacc atgcaaagtc tcagaatata caagtaccac tgggcttgta 360  
 ccatgttcag caacaccaac aacttttggg gacctcagag cagccaatgg ccaagggcaa 420  
 caacgacgcc gaattacatc tgtccagcca cctacaggcc tccaggaatg gctaaaaatg 480  
 tttcagagct ggagtgacc agagaaattg cttgctttag atgaactcat tgatagttgt 540  
 gaaccaacac aagtaaaaca tatgatgcaa gtgatagaac cccagtttca acgagacttc 600  
 atttcattgc tcctaaaga gttggcactc tatgtgcttt cattcctgga acccaaagac 660  
 ctgctacaag cagctcagac atgtcgctac tggagaattt tggctgaaga caaccttctc 720



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tggagagaga aatgcaaaga agaggggatt gatgaaccat tgcacatcaa gagaagaaaa 780
gtaataaaac caggtttcat acacagtcca tggaaaagtg catacatcag acagcacaga 840
attgatacta actggaggcg aggagaactc aaatctccta aggtgctgaa aggacatgat 900
gatcatgtga tcacatgctt acagttttgt ggtaaccgaa tagttagtgg ttctgatgac 960
aacactttta aagtttggtc agcagtcaca ggcaaagtgc tgagaacatt agtgggacat 1020
acaggtggag tatggtcatc acaaatgaga gacaacatca tcattagtgg atctacagat 1080
cggacactca aagtgtggaa tgcagagact ggagaatgta tacacacctt atatgggcat 1140
acttccactg tgcgttgat gcatcttcat gaaaaaagag ttggttagcgg ttctcgagat 1200
gccactctta gggtttggga tattgagaca ggccagtgtt tacatgtttt gatgggtcat 1260
gttcagcag tccgctgtgt tcaatatgat ggcaggaggg ttgtagtgg agcatatgat 1320
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aagctggtgt gtgcagttgg gagtcggaat gggactgaag aaaccaagct gctggtgctg 1860
gactttgatg tggacatgaa ggaattctgc agatatccag cacagtggcg gccgctcgag 1920
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<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: V5HIS tagged

homo sapien

<400> 25

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 20 25 30

Cys Leu Ser Met Ser Thr Leu Glu Ser Val Thr Tyr Leu Pro Glu Lys  
 35 40 45

Gly Leu Tyr Cys Gln Arg Leu Pro Ser Ser Arg Thr His Gly Gly Thr  
 50 55 60

Glu Ser Leu Lys Gly Lys Asn Thr Glu Asn Met Gly Phe Tyr Gly Thr  
 65 70 75 80

Leu Lys Met Ile Phe Tyr Lys Met Lys Arg Lys Leu Asp His Gly Ser  
 85 90 95

Glu Val Arg Ser Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu  
 100 105 110

Tyr Thr Ser Thr Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr  
 115 120 125

Phe Gly Asp Leu Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg  
 130 135 140

Ile Thr Ser Val Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met  
 145 150 155 160

Phe Gln Ser Trp Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu  
 165 170 175

Ile Asp Ser Cys Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile  
 180 185 190

Glu Pro Gln Phe Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu

195	200	205
Ala Leu Tyr Val Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala		
210	215	220
Ala Gln Thr Cys Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu		
225	230	235 240
Trp Arg Glu Lys Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile		
245	250	255
Lys Arg Arg Lys Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys		
260	265	270
Ser Ala Tyr Ile Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly		
275	280	285
Glu Leu Lys Ser Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile		
290	295	300
Thr Cys Leu Gln Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp		
305	310	315 320
Asn Thr Leu Lys Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr		
325	330	335
Leu Val Gly His Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn		
340	345	350
Ile Ile Ile Ser Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala		
355	360	365
Glu Thr Gly Glu Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val		
370	375	380

Arg Cys Met His Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp  
 385 390 395 400

Ala Thr Leu Arg Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val  
 405 410 415

Leu Met Gly His Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg  
 420 425 430

Arg Val Val Ser Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro  
 435 440 445

Glu Thr Glu Thr Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val  
 450 455 460

Tyr Ser Leu Gln Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp  
 465 470 475 480

Thr Ser Ile Arg Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr  
 485 490 495

Leu Thr Gly His Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn  
 500 505 510

Ile Leu Val Ser Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile  
 515 520 525

Lys Thr Gly Gln Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln  
 530 535 540

Ser Ala Val Thr Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser  
 545 550 555 560

Ser Asp Asp Gly Thr Val Lys Leu Trp Asp Leu Lys Thr Gly Glu Phe  
 565 570 575

Ile Arg Asn Leu Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val  
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Trp Arg Ile Arg Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser  
                           595                          600                          605

Arg Asn Gly Thr Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val  
                           610                          615                          620

Asp Met Lys Glu Phe Cys Arg Tyr Pro Ala Gln Trp Arg Pro Leu Glu  
                           625                          630                          635                          640

Ser Arg Gly Pro Phe Glu Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly  
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Leu Asp Ser Thr Arg Thr Gly His His His His His His  
                           660                          665

<210> 26

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<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: MYCHIS tagged

homo sapiens

<400> 26

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 tctgtgacat acctacctga aaaagggttta tattgtcaga gactgccaaag cagccggaca 180  
 cacggggggca cagaatcact gaaggggaaa aatacagaaa atatgggttt ctacggcaca 240  
 ttaaaaatga ttttttataa aatgaaaaga aagttggacc atggttctga ggtccgctct 300

ttttcttttg gaaagaaacc atgcaaagtc tcagaatata caagtaccac tgggcttgta 360  
 ccatgttcag caacaccaac aacttttggg gacctcagag cagccaatgg ccaagggcaa 420  
 caacgacgcc gaattacatc tgtccagcca cctacaggcc tccaggaatg gctaaaaatg 480  
 tttcagagct ggagtggacc agagaaattg cttgcttttag atgaactcat tgatagttgt 540  
 gaaccaacac aagtaaaaca tatgatgcaa gtgatagaac cccagtttca acgagacttc 600  
 atttcattgc tccctaaaga gttggcactc tatgtgcttt cattcctgga acccaaagac 660  
 ctgctacaag cagctcagac atgtcgctac tggagaattt tggctgaaga caaccttctc 720  
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 gtaataaaac caggtttcat acacagtcca tggaaaagtg catacatcag acagcacaga 840  
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 aacaagcatc agagtgtgtg gacctgttta cagttcaaca agaactttgt aattaccagc 1680  
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 gactttgatg tggacatgaa ggaattctgc agatatccag cacagtggcg gccgctcgag 1920  
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&lt;210&gt; 27

&lt;211&gt; 666

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

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&lt;223&gt; Description of Artificial Sequence: MYCHIS tagged

homo sapiens

&lt;400&gt; 27

Met Cys Val Pro Arg Ser Gly Leu Ile Leu Ser Cys Ile Cys Leu Tyr

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Cys Gly Val Leu Leu Pro Val Leu Leu Pro Asn Leu Pro Phe Leu Thr

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Cys Leu Ser Met Ser Thr Leu Glu Ser Val Thr Tyr Leu Pro Glu Lys

35 40 45

Gly Leu Tyr Cys Gln Arg Leu Pro Ser Ser Arg Thr His Gly Gly Thr

50 55 60

Glu Ser Leu Lys Gly Lys Asn Thr Glu Asn Met Gly Phe Tyr Gly Thr

65 70 75 80

Leu Lys Met Ile Phe Tyr Lys Met Lys Arg Lys Leu Asp His Gly Ser

85 90 95

Glu Val Arg Ser Phe Ser Leu Gly Lys Lys Pro Cys Lys Val Ser Glu

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Tyr Thr Ser Thr Thr Gly Leu Val Pro Cys Ser Ala Thr Pro Thr Thr

115 120 125

Phe Gly Asp Leu Arg Ala Ala Asn Gly Gln Gly Gln Gln Arg Arg Arg

130 135 140

Ile Thr Ser Val Gln Pro Pro Thr Gly Leu Gln Glu Trp Leu Lys Met

145 150 155 160

Phe Gln Ser Trp Ser Gly Pro Glu Lys Leu Leu Ala Leu Asp Glu Leu  
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Ile Asp Ser Cys Glu Pro Thr Gln Val Lys His Met Met Gln Val Ile  
 180 185 190

Glu Pro Gln Phe Gln Arg Asp Phe Ile Ser Leu Leu Pro Lys Glu Leu  
 195 200 205

Ala Leu Tyr Val Leu Ser Phe Leu Glu Pro Lys Asp Leu Leu Gln Ala  
 210 215 220

Ala Gln Thr Cys Arg Tyr Trp Arg Ile Leu Ala Glu Asp Asn Leu Leu  
 225 230 235 240

Trp Arg Glu Lys Cys Lys Glu Glu Gly Ile Asp Glu Pro Leu His Ile  
 245 250 255

Lys Arg Arg Lys Val Ile Lys Pro Gly Phe Ile His Ser Pro Trp Lys  
 260 265 270

Ser Ala Tyr Ile Arg Gln His Arg Ile Asp Thr Asn Trp Arg Arg Gly  
 275 280 285

Glu Leu Lys Ser Pro Lys Val Leu Lys Gly His Asp Asp His Val Ile  
 290 295 300

Thr Cys Leu Gln Phe Cys Gly Asn Arg Ile Val Ser Gly Ser Asp Asp  
 305 310 315 320

Asn Thr Leu Lys Val Trp Ser Ala Val Thr Gly Lys Cys Leu Arg Thr  
 325 330 335

Leu Val Gly His Thr Gly Gly Val Trp Ser Ser Gln Met Arg Asp Asn  
 340 345 350



Ile Ile Ile Ser Gly Ser Thr Asp Arg Thr Leu Lys Val Trp Asn Ala  
 355 360 365

Glu Thr Gly Glu Cys Ile His Thr Leu Tyr Gly His Thr Ser Thr Val  
 370 375 380

Arg Cys Met His Leu His Glu Lys Arg Val Val Ser Gly Ser Arg Asp  
 385 390 395 400

Ala Thr Leu Arg Val Trp Asp Ile Glu Thr Gly Gln Cys Leu His Val  
 405 410 415

Leu Met Gly His Val Ala Ala Val Arg Cys Val Gln Tyr Asp Gly Arg  
 420 425 430

Arg Val Val Ser Gly Ala Tyr Asp Phe Met Val Lys Val Trp Asp Pro  
 435 440 445

Glu Thr Glu Thr Cys Leu His Thr Leu Gln Gly His Thr Asn Arg Val  
 450 455 460

Tyr Ser Leu Gln Phe Asp Gly Ile His Val Val Ser Gly Ser Leu Asp  
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Thr Ser Ile Arg Val Trp Asp Val Glu Thr Gly Asn Cys Ile His Thr  
 485 490 495

Leu Thr Gly His Gln Ser Leu Thr Ser Gly Met Glu Leu Lys Asp Asn  
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Ile Leu Val Ser Gly Asn Ala Asp Ser Thr Val Lys Ile Trp Asp Ile  
 515 520 525

Lys Thr Gly Gln Cys Leu Gln Thr Leu Gln Gly Pro Asn Lys His Gln

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535

540

Ser Ala Val Thr Cys Leu Gln Phe Asn Lys Asn Phe Val Ile Thr Ser

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555

560

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565

570

575

Ile Arg Asn Leu Val Thr Leu Glu Ser Gly Gly Ser Gly Gly Val Val

580

585

590

Trp Arg Ile Arg Ala Ser Asn Thr Lys Leu Val Cys Ala Val Gly Ser

595

600

605

Arg Asn Gly Thr Glu Glu Thr Lys Leu Leu Val Leu Asp Phe Asp Val

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615

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Ser Arg Gly Pro Phe Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn

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655

Met His Thr Gly His His His His His His

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665

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/26820

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/47 C12Q1/68 C12N15/62 C07K16/18

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	E.J.A. HUBBARD ET AL.: "sel-10, a negative regulator of lin-12 activity in <i>Caenorhabditis elegans</i> , encodes a member of the CDC4 family of proteins." GENES & DEVELOPMENT, vol. 11, no. 23, 1 December 1997, pages 3109-3278, XP002097564 cited in the application see the whole document ----	
A	D. LEVITAN ET AL.: "Assessment of normal and mutant human presenilin function in <i>Caenorhabditis elegans</i> " PROC. NATL. ACAD. SCI. USA, vol. 93, December 1996, pages 14940-14944, XP002097562 cited in the application see the whole document ----- -/--	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

12 April 1999

Date of mailing of the international search report

22/04/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
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Fax: (+31-70) 340-3016

Authorized officer

Hix, R

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/26820

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>D. LEVITAN ET AL.: "Facilitation of lin-12-mediated signalling by sel-12, a <i>Caenorhabditis elegans</i> S182 Alzheimer's disease gene"</p> <p>NATURE, vol. 377, 28 September 1995, pages 351-354, XP002097563 cited in the application see the whole document</p> <p style="text-align: center;">---</p>	
A	<p>WO 97 11956 A (UNIV COLUMBIA ;GREENWALD IVA (US); LEVITAN DIANE (US)) 3 April 1997 cited in the application</p> <p style="text-align: center;">---</p>	
A	<p>M. SUNDARAM ET AL.: "Suppressors of a lin-12 Hypomorph define genes that interact with both lin-12 and glp-1 in <i>Caenorhabditis elegans</i>."</p> <p>GENETICS, vol. 135, November 1993, pages 765-783, XP002097565 cited in the application see the whole document</p> <p style="text-align: center;">-----</p>	

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/26820

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
W0 9711956 A	03-04-1997	AU 7251496 A	17-04-1997
		CA 2233297 A	03-04-1997
		EP 0854881 A	29-07-1998
<hr/>			

